Effect of Sibutramine on Macronutrient Selection in Male and Female Rats

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ABSTRACT

Sibutramine is a serotonin-noradrenaline reuptake inhibitor (SNRI) which has been shown to be a safe and effective weight-loss drug. The purpose of this study was to examine whether sibutramine has an effect on macronutrient selection in both female and male rats in addition to total food intake. Wistar rats of both sexes were divided into three groups and each group was offered a different set of three diets, each set included a carbohydrate-rich diet, a protein-rich diet and a fat-rich diet. Sibutramine (10mg/kg) was shown to consistently decrease carbohydrate and fat intake at all data points regardless of gender and diets. The effect of sibutramine on protein intake was diet and gender-specific. All doses of sibutramine decreased total food intake regardless of gender and diet group beginning at 6-h post-administration. In conclusion, sibutramine affected macronutrient selection and emphasis on dietary recommendations should be considered during therapy.

RESUME

La sibutramine est un inhibiteur de la recapture de la sérotonine et de la noradrénaline dont l'efficacité et l'innocuité ont été démontrées en tant que médicament pour la perte de poids. Le but de cette étude était d'examiner l'effet de la sibutramine sur la sélection des macronutriments en plus de la prise alimentaire totale chez des rats mâles et femelles. Des rats adultes des deux sexes (mâles et femelles) de souche Wistar, divisés en 3 groupes, ont reçu un choix simultané entre 3 diètes: une diète riche en sucre, une diète riche en gras, et une diète riche en protéine, avec un type de sucre, de gras et de protéine différent dans chacun des 3 groupes. La sibutramine a démontré un effet nutritionnel sur l'ingestion de sucre et de gras, nonobstant le sexe et la diète à une dose de 10mg/kg, tandis que l'effet sur l'ingestion de protéines était sensoriel. En conclusion, la sibutramine a affecté la sélection des macronutriments et le traitement thérapeutique devrait en conséquence inclure des recommandations nutritionnelles appropriées.

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The manuscript was written by the first author, under the supervision of the second author (thesis supervisor). It was revised by the second author, who provided valuable comments and suggestions to improve and finalize the manuscript. The second author also provided all the material, supplies and facilities that were needed to perform the study. This research was supported by a grant from Natural Sciences and Engineering Research Council of Canada held by the second author.

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LIST OF ABBREVIATIONS

ANOVA: analysis of variance

ATP: adenosine triphosphate

BBB: blood-brain barrier

C: Celsius

CHO: carbohydrate

DA: dopamine

5-HT: 5-hydroxytryptamine, serotonin

g: gram

h: hour

IP: intraperitoneal

kcal: kilocalorie

kg: kilogram

LNAA: large neutral amino acids

m^2: metres squared

mg: milligram

ml: millilitre

NA: noradrenaline

nmol: nanomole

NPY: neuropeptide Y

PRO: protein

PVN: paraventricular nucleus

SEM: standard error of the mean

SNRI: serotonin-noradrenaline reuptake inhibitor

Chapter 1

INTRODUCTION

In the ongoing battle against obesity, scientists have been struggling to find a solution. Current nutritional guidelines recommend a low-calorie diet and exercise to promote a weight loss (Health Canada, 2000). However, after initial weight loss, motivation seems to lapse after the expected plateau in weight-loss for the individual has been reached (Lean, 2001). This inevitable weight-loss plateau has spurred scientists to search for novel drugs. Drugs such as fenfluramine and fluoxetine were suggested as possible solutions, however, their potentially serious adverse effects has caused them to be removed from the market (Cannistra et al, 1997; Connoly et al., 1997; Mark et al., 1997; Rothman & Bauman, 2002). Sibutramine hydrochloride monohydrate, initially developed as an anti-depressant, was found to cause weight-loss in depressed, obese test subjects (Kelly et al., 1995). Since then, it has been shown that sibutramine is a safe and effective weight-loss drug that can be administered for up to 2 years without serious side effects (Bray et al., 1998; James et al., 2000). At present, sibutramine has been approved for use, in the treatment of obesity, in nearly 40 countries since its official introduction in February 1998 by Knoll Pharmaceuticals, a subsidiary of Abbott Laboratories (Luque & Rey, 2002).

Sibutramine is primarily a serotonin-noradrenaline reuptake inhibitor (SNRI). A reuptake inhibitor inhibits the neuronal uptake of neurotransmitters and prolongs the duration of responses to both exogenous and neuronal release, in this case, of serotonin (5-HT) and noradrenaline (NA). Sibutramine has also been shown to block the re-uptake

of dopamine (DA) but at about a 3-fold lower potency when compared to 5-HT and NA (Luscombe et al., 1989). More recent studies suggest that sibutramine increases extracellular DA concentrations at similar levels to 5-HT in an animal model (Balcioglu & Wurtman, 2000; Rowley et al., 2000). Serotonin, an indole amine and neurotransmitter, is recognised to have an influence on food intake and macronutrient selection (reviewed by Leibowitz & Alexander, 1998 and Thibault & Booth, 1999). Noradrenaline, a catecholamine and neurotransmitter, has also been shown to have an effect on food intake but its effects on macronutrient selection are not clear (reviewed by Thibault & Booth, 1999). Dopamine and its agonist, amphetamine, have been shown to have an effect on food intake but similar to NA its effects on macronutrient selection are not clear (reviewed by Thibault & Booth; Yang et al., 1997).

The effects on feeding of 5-HT agonists have been extensively studied. For example, fluoxetine is similar to sibutramine in that it is also a 5-HT reuptake inhibitor while fenfluramine and dexfenfluramine are 5-HT-releasing agents (reviewed by Lean, 2001). Noradrenaline agonists such as clonidine and LY368975 have been studied for their effects on food selection as well (Leibowitz et al., 1985; Shor-Posner et al., 1988; Currie, 1993; Currie & Wilson, 1992; Gehlert et al., 1997). As a 5-HT-NA reuptake inhibitor, it is expected that sibutramine, in addition to affecting total food intake, could also affect food selection.

Chapter 2

LITERATURE REVIEW

2.1-Food Intake and Macronutrient Selection

2.1.1-Feeding Behaviour

The behaviour of eating and drinking is based on the control of "gathering and ingesting substances by physical and social exteroceptive stimuli and interoceptive signals from the gastrointestinal tract, metabolism of tissue, and the circulation of substrates and hormones" (Thibault & Booth, 1999). This behaviour can be explained by the neural distribution of microanatomical electrical processes and chemical transmissions, which surround the central nervous system with signals towards and from areas of stimulation to then return to skeletal, autonomic and neuroendocrine stimulants. These outputs influence specifically the sensory and motor controls of the selection and placement of foods into the mouth (Thibault & Booth, 1999).

2.1.2-Macronutrient Selection

2.1.2.1-Serotonin Agonists and Antagonists and Macronutrient Selection

Serotonin appears to be responsible for various behaviours and functions linked to its ability to generally inhibit behavioural responding and modulate motor behaviour (Lucki, 1998). These behaviours and functions include reduced anxiety and libido as well as, optimised vigilance, sleep, the control of eating patterns and the intake of specific macronutrients such as carbohydrate (Leibowitz et al., 1989; Thibault & Booth, 1999; Portas et al., 2000; Ressler & Nemeroff, 2000; Rosen et al., 1999; Strüder & Weicker, 2001). Previous research has demonstrated that an intake of pure carbohydrate

increased the synthesis of 5-HT in rats and that an intake of pure protein had no effect (Ashcroft et al., 1965). Research has also shown that 5-HT has an inhibitory effect on food intake.

Many have examined the physiological effect of 5-HT agonists on feeding behaviour. Treatment with 5-HT agonists, such as fenfluramine, promotes satiety while also influencing energy expenditure with the activation of brown fat tissue thermogenesis (Lupien & Bray, 1985; Rothwell & Stock, 1987). Studies have been completed on 5-HT agonists and macronutrient selection. Wurtman and Wurtman (1977) investigated the effects of dl-fenfluramine and fluoxetine, in male rats 21-48 days old (strain not reported) trained to consume their daily food intake in an 8-hour dark period. The animals were given the choice between two isocaloric diets either with high carbohydrate-low protein (80% dextrin and 5% casein) or low carbohydrate-high protein (40% dextrin and 45% casein) content. Dl-fenfluramine, at a dose of 2.5mg/kg injected into the intraperitoneal (IP) at early dark, resulted in the depression of total intake, 1-4 hours post-injection. When the animals were presented instead with two diets differing only in carbohydrate content (diet 1: 24% sucrose, 24% dextrin, 24 % dextrose, and 23% casein; diet 2: 48% sucrose, 12% dextrin, 12% dextrose, and 23% casein) no difference in total intake was noted after 2.5mg/kg dl-fenfluramine administration into the IP at early dark. Shor-Posner et al. (1986) examined the effect of dl-norfenfluramine, dl-fenfluramine, quipazine and cyproheptadine on macronutrient selection in albino male, Sprague-Dawley rats weighing approximately 450 grams. Both dl-norfenfluramine and dl-fenfluramine are 5-HT releasing agonists while quipazine is a selective 5-HT_3 agonist and

cyproheptadine is a 5-HT antagonist (Shor-Posner et al, 1986). The animals were maintained on a constant 12:12 light-dark cycle with the lights at 6h00. When offered a choice between two isocaloric diets (82.7% dextrin and 82.7% casein), after being food deprived for the first 2-h of the dark phase, 150nmol of dl-norfenfluramine, injected centrally into the paraventricular nucleus (PVN) at early dark, caused a significant decrease in the high-carbohydrate diet consumption 2-h after the presentation of the diets (Shor-Posner et al, 1986). When offered a choice between three macronutrient-specific diets (CHO: 37% sucrose, 28% dextrin, and 28% cornstarch; PRO: 93% casein; FAT: 86% lard), after a 2-h food deprivation period at the onset of the dark phase, 2mg/kg of dl-fenfluramine, injected into the IP at early dark, caused a decrease in total intake, as well as the protein diet with a marked decrease found in both the carbohydrate and fat diets consumption 2-h after the presentation of the diets (Shor-Posner et al, 1986). Similarly, quipazine, at a dose of 2mg/kg, injected into the IP at early dark, caused a decrease in all three macronutrient-specific diets with the biggest decrease in the intake of the fat diet measured 2-h after the presentation of the diets following a 2-h food deprivation period (Shor-Posner et al, 1986). Cyproheptadine, at a dose of 2mg/kg, injected once again into the IP at early dark, resulted in an increase in both total intake, as well as all three macronutrient-specific diets with more notable increases in the carbohydrate and fat diets consumption measured 2-h after the presentation of the diets following a 2-h food deprivation period (Shor-Posner et al, 1986). Kim and Wurtman (1988) investigated the effect of 2.5 and 3mg/kg CGS 10686B, a 5-HT re-uptake inhibitor, as well as 3 and 6mg/kg dl-fenfluramine and 1.5 and 7.5 mg/kg fluoxetine on the choice between two isocaloric-isocarbohydrate diets. These diets were mixed with 1

litre of 4% agar solution/kg of dry ingredients (diet 1: 5% casein, 40% dextrin, 23% vegetable shortening, and 24.9% cellulose; diet 2: 45% casein, 40% dextrin, 6.1% vegetable shortening, and 2.7% cellulose) and offered to 100-120 gram male Sprague-Dawley rats. These animals were maintained on a reversed light-dark cycle with the dark phase being from 10h00-22h00. The animals were given a 4-h food deprivation period beginning 1-h before the onset of the dark phase. All three drugs, injected into the IP at early dark, 30 minutes before the presentation of food, suppressed the intake of diet 1, the low protein diet, measured 90 minutes after the food presentation, hence decreasing overall carbohydrate consumption. Luo and Li (1990) conducted a similar experiment with male Wistar rats weighing 230 ±10 grams, maintained on a 12:12-h light-dark cycle with lights on at 07h00, and having access to food only in the dark phase. These animals were treated with 0.5, 1.0, and 1.5mg/kg dexfenfluramine, 2mg/kg fluoxetine, or 1mg/kg RU 24969, all three being 5-HT agonists. After being injected with one of the three drugs into the IP at late light, the rats were offered two isocaloric diets that differed in carbohydrate and protein content (high carbohydrate-low protein diet: 78% cornstarch and 5% casein; low carbohydrate-high protein diet: 38.5% cornstarch and 45% casein). All three drugs caused a decrease in the high carbohydrate-low protein diet measured at 1-h and 2-h after the presentation of food. The effect was most prominent at lower doses (dexfenfluramine, fluoxetine, and RU 24969: 0.5, 2.0, and 1.0mg/kg, respectively). Luo and Li (1991) then went on to repeat the same experiment examining the effect of repeated administration of these three drugs (1.5mg/kg of dexfenfluramine and RU 24969, and 3mg/kg of fluoxetine) for six consecutive days on diet selection. The results were the same with a decrease in the high carbohydrate-low protein diet intake after 2-h

of feeding, which was sustained upon repeated measure. Weiss et al. (1990) investigated the effect of injecting 3.1, 6.2, 12.5, and 25nmol d-norfenfluramine into the PVN at both early (1st h), middle (6th h), and late (11th h) dark after dark onset, as well as into the ventromedial nucleus at a dose of 1.6nmol at early dark using 280-300 gram Sprague-Dawley male albino rats. Weiss et al. (1990) also examined the effect of injecting 0.06, 0.13, 0.25, 0.5, and 1mg/kg d-fenfluramine into the IP at early, middle, and late dark in the same type of rats. The animals were maintained on a constant 12:12-h light-dark cycle with lights off at 10h00 for tests at the onset and the middle of the dark phase and lights on at 14h00 for tests at the end of the dark phase. The rats were offered the choice between three macronutrient-specific diets (CHO: 37% sucrose, 28% dextrin, and 28% cornstarch; PRO: 93% casein; FAT: 86% lard) and had *ad libitum* access to the food. D-norfenfluramine caused a dose-dependent decrease in carbohydrate consumption without affecting both protein and fat intake at the beginning of the dark phase, 1-h post-injection. D-fenfluramine caused a dose-dependent reduction in carbohydrate intake with the largest effect occurring at lower doses (0.06-0.5mg/kg) at early dark, 2-h post injection. Weiss et al. (1991) then went ahead and studied the effect of injecting fluoxetine into the PVN or into the IP at the early and late dark phase. Once again, the male, albino, Sprague-Dawley rats, weighing 280-300 grams, were fed three pure macronutrient diets (CHO: 37% sucrose, 28% dextrin, and 28% cornstarch; PRO: 93% casein; FAT: 86% lard) *ad libitum*. The animals were maintained on the same 12:12-h light-dark cycles as mentioned in the previous experiment. Fluoxetine, both into the PVN (3.2-100nmol) and into the IP (.6-10mg/kg), decreased the intake of the carbohydrate diet selectively and dose-dependently. This result occurred at a dose as low as 3.2nmol into the PVN and at

0.6mg/kg into the IP, only during the first hour after injection at the early dark phase. Leibowitz et al. (1993) examined the effect of d-norfenfluramine and fluoxetine (both at doses 3.1-25nmol), and metergoline (0.03-1.0mg/kg), a 5-HT receptor antagonist. These drugs were injected into the PVN immediately before the dark phase of 250-300 gram male Sprague-Dawley rats offered three pure macronutrient diets (CHO: 37% sucrose, 28% dextrin, and 28% cornstarch; PRO: 93% casein; FAT: 86% lard) *ad libitum*. The animals were maintained on a 12:12-h light-dark cycle with lights off at 15h30. The results were the same in that both 5-HT agonists decreased the intake of carbohydrate at the onset of the dark phase, 1-h post-injection. Metergoline stimulated feeding by increasing the intake of carbohydrate at the beginning of the dark phase, 1.5-h post-injection. Paez and Leibowitz (1993) examined the effect of 10mg/kg of fluoxetine on the 250-300 gram male Sprague-Dawley rat's *ad libitum* choice of three macronutrient-specific diets (CHO: 37% sucrose, 28% dextrin, and 28% cornstarch; PRO: 93% casein; FAT: 86% lard) two hours post-injection in the IP immediately prior to dark onset. The animals were maintained on a reversed 12:12-h light-dark cycle (specific times not reported). Fluoxetine decreased total food intake and specifically carbohydrate intake. Mok et al. (2000) examined the effect of 2.5, 5.0, and 7.5mg/kg of quipazine injected into the IP on adult male and female Wistar rats' selection of different macronutrient diets. The rats, weighing 225-250 grams, were divided into three groups. The animals were maintained on a constant 12:12-h light-dark cycle with lights on at 08h00. Each group received a different set of three choices of isocaloric macronutrient pure diets: Group 1 (92.24% casein, 92.9% cornstarch, and 48.78% safflower oil diets), Group 2 (92.9% egg protein, 72.26% cornstarch/20.64% sucrose, and 48.78% lard diets), Group 3 (92.24%

casein hydrolysate, 91.9% maltose dextrin, and 48.78% butter diets). Each set contained diets that differed sensorily from their equivalent counterparts in the other sets. A 4-h food deprivation period between 16h00-20h00 preceded the IP injections at dark onset (20h00). Food intake was measured at 2-h and 12-h post-injection. The intake of cornstarch-containing diets was reduced in both male and female rats at 12-h post-injection with doses 2.5 and 7.5mg/kg.

2.1.2.2-Noradrenaline, Food Intake and Macronutrient Selection

Noradrenaline is produced in the adrenal medulla, as well as in the brain, and is derived from tyrosine, a semi-essential amino acid (Nelson, 1995). Brain/central nervous system NA helps to initiate and/or maintain normal eating behaviour as well as influencing sleep, pain, and sexual function (Stanley et al., 1989; Gottesmann, 1999; Rampin, 1999; Baron, 2000; Bond, 2001). In 1975, Leibowitz examined the effect of NA (3.1-100nmol) injection on drinking and feeding in satiated 350-400 gram albino male Sprague-Dawley rats. The noradrenaline was injected directly into the hypothalamus (time of day not reported) and this resulted in a dose-dependent increase in intake of water and food which occurred at a dose as low as 3.1nmol. The size of the meal (grams of lab chow pellets at 60-minute intervals and millilitres of water intake at 5-minute intervals) ingested following NA injection was similar to the amount of food eaten per day. Leibowitz et al. (1984) then went on to examine the effect of chronic NA (20nmoles/injection given 4 times/day) injection into the PVN of 375-400 gram albino male Sprague-Dawley rats. The animals were maintained on a 12:12-h light-dark cycle with lights on at 06h00. With chronic multiple daily injections, there occurred a

stimulation of eating after each injection and resulted in a significant increase in total daily food intake (lab chow pellets or "milk-mash" consisting of chow and undiluted sweetened condensed milk) 1-h post-injection. This result occurred with both a food restriction schedule where food was available only during the light phase and a food-satiated schedule where food was available *ad libitum*. Shor-Posner et al. (1985) also studied the effect of NA (40nmoles) on meal patterns and food intake in 450-gram albino male Sprague-Dawley rats. The animals were maintained on a 12:12-h light-dark cycle with lights on at 05h00 and offered, *ad libitum*, a sweetened powdered diet consisting of 80% Purina lab chow powder mix with 20% sucrose. When noradrenaline was injected into the PVN in both the light (10h00) and dark (17h00) phases of the day the result was hyperphagia, meaning the stimulation of a larger intake of food per meal (measured by a PDP computer and defined as a minimum of 10 licks occurring within 10 seconds and separated from other meals over a 24-h period). Bishop et al. (2000) also examined the effect of 20nmol NA on *ad libitum* food intake (Rodent Diet 5001: 28% protein, 12% fat, and 60% carbohydrate) in 275-300 gram albino male Sprague-Dawley rats. The animals were maintained on a 12:12-h light-dark cycle with lights on at 05h00. Noradrenaline was injected directly into the PVN at the dark-onset and resulted in a relatively immediate, brief feeding, 2-h post-injection, that was not associated with changes in nutrient metabolism and did not increase with cues brought on by food deprivation. Pal et al. (2001) examined the effect of 0.1-2 micrograms of NA injected into the nucleus caudatus on feeding and drinking in male rats (strain and age not available). The total daily intakes of food and water, offered *ad libitum*, were measured after NA injection and found to have increased significantly with increasing doses (0.1, 0.5, 1.0, and 2.0

micrograms). Therefore, these above-mentioned studies help to conclude that NA potentiates food intake and induces hyperphagia in rats. This is in opposition to the effects of 5-HT as discussed in the section on 5-HT and the brain. At the receptor level in the brain, NA has different actions at the α_1 and α_2-receptors. In the PVN, NA induces food intake when acting on α_2-receptors but inhibits food intake when acting on α_1-receptors (Leibowitz, 1988; Wellman & Davies, 1991).

Effects of NA on macronutrient selection are not clear though. For example, acute and chronic infusion of NA (40nmoles, and 5nmoles every 30 minutes over a 14-day period, respectively) into the PVN on the selection among three macronutrient pure diets (CHO: 37% sucrose, 28% dextrin, and 28% cornstarch; PRO: 93% casein; FAT: 86% lard) fed *ad libitum* to 350 gram albino male Sprague-Dawley rats, resulted in a strong and selective increase in carbohydrate intake with a slight decrease in protein and no change in fat intake (Leibowitz et al., 1985). Tempel and Leibowitz (1990) injected 40nmoles of NA into the PVN of 250-300 gram adult male Sprague-Dawley rats offered a choice among three pure macronutrient diets (CHO: 28% dextrin, 37% cornstarch, and 37% sucrose; PRO: 93% casein; FAT: 96% lard) *ad libitum*. The animals were maintained on a reversed 12:12-h light-dark cycle with lights on at 22h00. The researchers reported an increase in carbohydrate intake at the beginning of the dark period, 1-h post-injection. Currie (1993), using lean (average weight was 27.2 grams) and obese (ob/ob; average weight was 56.9 grams) mice examined the effect of 20-80nmol of NA, injected into the PVN immediately prior to dark onset, on macronutrient selection among three single-energy source diets (CHO: 43.9% dextrin and 43.9 %

starch; PRO: 86.3% casein; FAT: 70.5% lard and 10% corn oil) offered *ad libitum*. The animals were maintained on a 12:12-h light-dark cycle with lights on at 05h00. Noradrenaline injection resulted in an increase in preference for carbohydrate diet intake and a reduction in the proportional intake of the protein and fat diets 2-h post-injection in both lean and obese (ob/ob) mice. The results further indicated that the effect of hyperphagia was dose dependent (40-80nmol and not at 20nmol). Although these studies strongly suggest a role of NA on macronutrient selection other studies did not support these findings (as reviewed by Rowland et al., 1996; Thibault and Booth, 1999).

NA agonists, such as clonidine and LY368975, have also been studied to determine their effects on macronutrient selection and food intake. Clonidine, a NA reuptake inhibitor, when injected into the IP (25 micrograms/kg in mice) or the PVN (0.5mg/kg in rats and 5-20nmol in mice) in male albino Sprague-Dawley rats or lean and obese ob/ob mice with a choice of three pure macronutrient diets (CHO: 37% sucrose, 28% dextrin, and 28% cornstarch; PRO: 93% casein; FAT: 86% lard for the rats and, CHO: 43.9% dextrin and 43.9 % starch; PRO: 86.3% casein; FAT: 70.5% lard and 10% corn oil for the mice). In one of the experiments, the mice were restricted to a 6-h feeding period between 09h00-15h00 (Currie & Wilson, 1992). The injection of clonidine caused the same hyperphagia and carbohydrate preference as NA between 1-3-h post-injection (Shor-Posner et al., 1988; Currie & Wilson, 1992; Currie, 1993). The selective NA reuptake inhibitor LY368975, when injected subcutaneously at doses 0.1-10mg/kg in male Sprague-Dawley rats (160-200 grams), suppressed food intake in food-deprived rats (at 3 and 10mg/kg dose with intake measured 1-2-h post-food presentation)

and decreased a preference for sweetened condensed milk (at all doses tested 0.3-3mg/kg, 1-h post-injection) diluted 1:3 with water (Gehlert et al., 1998). The animals trained to eat the sweetened milk were maintained on a light-dark cycle with the lights on at 13h00 and the lights off at 01h00. These rats were food restricted for 1-h post-injection then their intake of the milk was measured 15 minutes after the presentation of the milk. The animals in the food deprivation experiment were maintained on a 12:12-h light-dark cycle with lights on at 07h00 and fasted for 18-h prior to drug injection and then food was presented 15 minutes post-injection (Gelhert et al., 1998). Different effects of clonidine and LY368975 can be explained by the fact that clonidine is a known α_2-receptor agonist, which is known to cause an increase in food intake. The exact mechanism of the LY368975 effect is unknown but it could be due to the fact that it is an α_1-receptor agonist. From the above mentioned studies, it is difficult to rule out the effects of the sensory characteristics of the diets on macronutrient selection since only one set of diets was used in each study. However, Thibault and Booth (1999) examined all studies conducted with NA in various laboratories with varied dietary conditions and no consistent effect was found.

2.1.2.3-Dopamine, Food Intake and Macronutrient Selection

Although Luscombe et al. (1989) found the effect of sibutramine on dopamine (DA) to be three-fold less than the effect on 5-HT and NA, two recent studies have reported conflicting results. Balcioglu and Wurtman (2000) examined the effect of sibutramine on the concentration of DA in the rat striatal and hypothalamic extracellular

fluid. Sibutramine was found to cause a dose-dependent increase in hypothalamic DA concentration that was as great as the increase in 5-HT concentrations in both striatal dialysates and the hypothalamus. This effect was seen at dose of 5 and 10mg/kg but not at 2.0mg/kg. Another similar study conducted by Rowley et al. (2000) found that sibutramine, at a dose of 6.0mg/kg, injected into the nucleus accumbens resulted in a modest and prolonged increase in extraneuronal DA. This effect, however, was not equivalent to the greater effects caused by d-amphetamine and phentermine, both indirect NA and DA agonists at lower doses (0.5 and 1.3mg/kg, respectively). These recent studies justify a review of the effect of DA on food intake and macronutrient selection. Dopamine could contribute to the effect of sibutramine on food intake and macronutrient selection. Dopamine and its agonists, such as amphetamine, have been shown to have an effect on food intake. Dopamine, along with affecting eating behaviour, also has an effect on sexual function, copulatory behaviour, stress management, depression, motivation and the control of motor pathways (Laverty, 1978; Di Chiara, 1995; Cabib & Puglisi-Allegra, 1996; Giuliano & Allard, 2001). Dopamine's effect on food intake in rats has been extensively studied. Studies have mainly focused on the effects of amphetamine, a non-selective DA agonist. The effect on food intake appears to be dose dependent with food intake increasing at doses less than 1.0mg/kg and amphetamine has an anorectic effect at doses higher than 1.0mg/kg (Winn et al, 1982, as cited in Vaccarino, 1996). The hypothalamic region of the brain seems to be responsible for the anorexic effect of amphetamine while the caudate and the nucleus accumbens regions are responsible for increases in food intake after administration of amphetamine or DA (Pal and Thombre, 1993, as cited in Vaccarino, 1996). Yang et al (1997) examined the effect

of bilateral hypothalamic DA infusion in obese male Zucker rats. Dopamine, at a dose of 11mg/ml with 0.5 microliter/hour, was administered for 13 days into two osmotic minipumps resulting in a daily dose of 0.264mg. Meal size, number of meals, and food intake were continuously measured before, during and after DA injection. Food intake, as a result of a significant reduction in meal size, was decreased significantly.

Amphetamine has been examined for its possible effect on macronutrient selection. Kanarek et al. (1981) examined the effect of d-amphetamine sulfate, injected into the IP at early dark, on the intake of three macronutrient-specific diets (CHO: 56.8% cornstarch, 27.4% dextrin, and 9.8% sucrose; PRO: 93.9% casein; FAT: 87.3% vegetable shortening) in 225.9 gram female Sprague-Dawley rats. The rats were restricted to a daily 6-h period of food access during the dark phase. Two hours after administration of the drug (0.5, 1, and 2mg/kg) both carbohydrate and protein-rich diet intakes were decreased but returned to control values at the end of the 6-h feeding period. The fat-rich diet was initially decreased as well but remained lower than control values for the entire 6-h feeding period. Orthen-Gambill and Kanarek (1982) examined the effect of 0.5-2mg/kg of d-amphetamine on the intake of both a regime of three macronutrient-specific diets with a high-caloric fat ration (CHO: 56.9% cornstarch, 27.5% dextrin, and 9.8% sucrose; PRO: 94.1% casein; FAT: 83% vegetable fat and 4.4% safflower oil) and a regime with three isocaloric macronutrient-specific diets (CHO: 56.9% cornstarch, 27.5% dextrin, and 9.8% sucrose; PRO: 94.1% casein; FAT: 39.4% vegetable fat and 2.1% safflower oil). These diets were offered to 175-gram female Sprague-Dawley rats for an 8-h period during the light phase. Throughout the 8-h feeding period, d-amphetamine,

injected into the IP at early dark, decreased caloric intake but most significantly during the first 2-h post-injection for both regimes. Amphetamine selectively decreased the caloric intake from the high-caloric fat ration diet in the first regime and the drug caused a reduction of caloric intake from all three isocaloric diets. McArthur and Blundell (1983) examined the effect of 0.5-2mg/kg of d-amphetamine administered by IP injection on protein and carbohydrate self-selection in the rat (two isocaloric diets with 5% casein or 45% casein, carbohydrate content was not reported). D-amphetamine decreased intake of the protein-rich diet 1-h post-injection, in adult and adolescent Lister Hooded male rats that had been adapted to *ad libitum* access to food or to food access being restricted to an 8-h period beginning at the onset of the dark phase. Leibowitz et al. (1986) conducted a study examining the effect of 150nmol of either d-amphetamine or DA, injected into the lateral perifornical hypothalamus at early dark or 5mg/kg of d-amphetamine injected into the IP at early dark, on the intake of 350-400 gram male Sprague-Dawley rats having been food deprived for 2-h. The rats, in one of the experiments were offered a simultaneous choice among three macronutrient-specific diets (CHO: 37% sucrose, 28% dextrin, and 28% cornstarch; PRO: 93% casein; FAT: 86% lard) with central or peripheral injection of d-amphetamine. In another two experiments, the rats were offered one of two sets of two different diets (Set 1: diet 1: 26% casein, 52% dextrin, and 15% corn oil; diet 2: 6% casein, 67% dextrin, and 20% corn oil; Set 2: PRO-FAT diet: 82.7% casein and 10% corn oil; CHO-FAT diet: 82.7% dextrin and 10% corn oil) with central injection of both d-amphetamine and DA. Both d-amphetamine and DA central injections resulted in a significant decrease in protein intake 1-h post-injection while d-amphetamine, injected peripherally caused a mild decrease of fat intake 2-h post-

injection. The results on amphetamine's effect on macronutrient selection are inconclusive because an affect of sensory of motor processes related to the individual diets cannot be ruled out since only one set of mixed or macronutrient-specific diets were used in previous studies.

The effects of DA receptor-selective drugs on feeding behaviour are not yet completely characterised. Terry (1996) reviewed the evidence to date and found that D_1-type agonists (selective for D_1 and D_5 receptor subtypes) consistently cause anorexia but the role of behavioural responses and receptor-specificity of this effect remains unclear. D_1-type antagonists can also cause a decrease in food intake but this effect seems linked to changes in motor behaviour. D_2-type agonists and antagonists (selective for D_2, D_3, and D_4 receptor subtypes) appear to cause a biphasic effect on food intake with high doses decreasing food intake and low doses increasing food intake. The cause of these effects is still not well understood. Terry et al. (1995) proposed a scheme that D_1-type agonists may produce a satiety signal that promotes meal termination and D_2-type agonists might modify the manner of eating. This scheme does not, however, include the effects of the antagonists.

2.1.2.4-Rat Macronutrient Selection Pattern

The individual laboratory rat is influenced by many factors during macronutrient selection such as age, gender, and individual personal preferences. The time of day (light/dark phases) and food deprivation have also been shown to affect macronutrient selection in the rat.

Leibowitz et al. (1991) demonstrated that albino female Sprague-Dawley rats had a preference for carbohydrates when offered a choice among three isocaloric macronutrient-specific diets (CHO: 37% sucrose, 28% dextrin, and 28% cornstarch; PRO: 93% casein; FAT: 86% lard) during their entire lifespan with a peak at puberty and then a decline with stabilisation. Albino male Sprague-Dawley rats preferred protein up until and peaking at the age of puberty when offered a choice among the same three isocaloric diets. At puberty there was a marked decline and protein intake levelled off while carbohydrate intake increased and then stabilised. Also, male rats seemed to eat more fat than female rats. During the first week after weaning, both genders seemed to have a moderate preference for fat, which after puberty had a dramatic increase to 25% of total intake, which remained for the rest of the rats' lifespan. Male rats also showed a stronger preference for fat when determining total intake and weight gain. Both male and female rats were found to have the same overall intake for the first week after weaning but as time progressed the male rats consumed almost 50% more than the female rats with a relative increase in bodyweight for the male rats. This could be explained by studies suggesting that male rats have less control over food intake, energy intake, and bodyweight when compared to female rats (Nance et al, 1977; Bjorntorp 1989; Richard & Rivest, 1989). Veyrat-Durebex et al. (1998) suggested an age-related shift of a preference for carbohydrates to fat and an overall decrease in protein for both males and females but shifts were more pronounced in male rats.

Along with age and gender differences in macronutrient selection, rats have been shown to exhibit preferences for pure macronutrient diets. Shor-Posner et al. (1991) characterised three subpopulations of rats in terms of their macronutrient preferences. These subgroups, namely, high carbohydrate which made up 50 % of the population, high protein, with 20% of the population, and high fat, taking up the remaining 30% of the population, also differ in their body weight (high fat > high protein > high carbohydrate). These preferences were most notable at the onset of the nocturnal (active) cycle.

Rats consume most of their food during the active period of a diurnal cycle, and are able to regulate their intake in relation to their needs on a daily basis. Freely feeding adult male rats consume most of their intake at the beginning and the end of the dark period or active phase (Tempel et al., 1989). In terms of macronutrient selection, there is a circadian variation in which male rats seem to prefer a carbohydrate diet at the beginning of the dark phase with a shift towards protein and fat at the end of the dark phase (Tempel et al, 1989, Lax et al., 1998). Leibowitz et al. (1991) noticed that female rats differed in their diurnal rhythm by consuming a larger portion of their carbohydrate diet and fat in the light phase when compared to male rats. Hence, the female rats, after puberty, consumed a significantly lower percentage of carbohydrate and fat in the dark period compared to the male rats.

Another factor that has been studied as affecting the macronutrient selection in rats is food deprivation. After a 2-h food deprivation at the beginning of the dark phase, the animals consumed the same amount of food in 1-h that they normally would have

consumed in three hours with a notable preference for carbohydrate and fat. Rats did not seem to be able to adjust for protein intake (Tempel et al., 1989). Lax et al. (1998) studied the effects of repeated short fasting (3-h) for 15 days at the beginning or the end of the dark phase, and found that rats were able to compensate completely for energy intake over the 24-h period. When deprived at the beginning of the dark phase, the reorganisation of intake took place in the 6-h following deprivation. Also, rats expressed a preference for fat following fasting when offered a choice among three macronutrient-specific diets (CHO: 85% cornstarch and 8% commercial grade sucrose; PRO: 92.5% casein; FAT: 91% lard and 2% sunflower oil).

When rats were subjected to food restriction (in order to maintain 85% normal body weight), their preference for a sweet taste (sucrose) diminished while preference for fat (corn oil) increased significantly. This suggests that nutritional status may affect food preferences (Sclafani & Ackroff, 1992; Lucas & Sclafani, 1996).

2.1.3-The Proper Experimental Design

Thibault and Booth (1999) stated that in order to assess whether the selection of a diet to be based on the presence of a specific macronutrient, the selection process must be controlled for the sensory characteristics specific to that macronutrient. However, the eater must be able to detect the presence of only one macronutrient in that diet in order to select that diet specifically because of the presence of that macronutrient. Without a sensory cue to indicate the presence of only one macronutrient, the ingestive behaviour cannot be a result of selective intake. If only one set of diets are mixed, such as a choice

among a high-carbohydrate, a high-protein and a high-fat diet, macronutrient-specific selection becomes problematic since the macronutrient-specific sensory cues can confound the choice within the diets. There are sensory characteristics that are inherent to macronutrients such as different smells, taste, textures/mouthfeels and appearance. For example, rats can discern the difference among egg, cow's milk and soybeans based on their different smells (Deutsch et al., 1989). Isolated fat has a particular glossy sheen, which is not usually present in either carbohydrate or protein. The three macronutrients are also very different in texture. Carbohydrates are crystals, rubbery gels or glasses. Proteins are usually sticky gels, and fats are oily pastes or liquids. The mouthfeel of each macronutrient is also different when combined with water. Rats are known to have sensory preferences to oily or sweet and crisp foods, and this may influence the selection or amount eaten regardless of the macronutrient content of the diet (Hamilton, 1964).

Dl-fenfluramine, for example, may have different effects on the relative preferences for the sensory characteristics of the diets offered (Baker & Booth, 1990). If only a single set of sensorily discernible diets are used in a study, then the results do not provide evidence for nutrient selection. The effects of the drug may be a result of the actions on the sensory or motor systems in the rat rather than on its behaviour controlled by the macronutrients. In other words, there is the possibility that the food intake is influenced by the nutrient content of the diet, and not by the action of the drug on macronutrient selection. The scientific principle behind this argument is that the make-up of the dietary choices (carbohydrate, protein and fat-rich diets) is an important element when determining the effect of a drug on macronutrient selection, as well as possible

mechanisms (Thibault & Booth, 1999). The experimental design should consist of two or more diet sets that are equal in energy content but that differ considerably in sensory characteristics. Only with this type of design can it be determined that the drug is acting on feeding behaviour rather than on sensorimotor pathways (Booth &Thibault, 2000). In the section on 5-HT agonists and antagonists and macronutrient selection, the criteria are met by comparing so many studies. One is able to examine the effect of the drugs such as d-norfenfluramine and fluoxetine on more than one set of diets and conclude that these drugs do reduce the intake of carbohydrate diets (Thibault & Booth, 1999).

2.2-Sibutramine and Feeding

2.2.1-Effects on Serotonin and Noradrenaline

The justification of a possible effect of sibutramine, a 5-HT-NA reuptake inhibitor, on macronutrient selection is founded partly on studies conducted with 5-HT or NA and their effects on the brain and how those effects relate to feeding. Research has focused on the hypothalamic PVN which is believed to play an important role in feeding behaviour (refer to appendix A1 for a picture of the hypothalamus and the location of the PVN). The hypothalamus is thought to be the structure responsible for receiving and integrating input from various factors that represent an animal's nutritional status. The hypothalamus then interprets the input and sends signals to adjust the nutrient intake and thus regulates energy balance (Leibowitz et al, 1990).

2.2.2-Mechanisms of Neuronal Transmission and Reuptake Inhibition

These signals from the hypothalamus, which are responsible for adjusting nutrient intake and regulating energy balance, take place at the level of the neuron. They involve neurotransmitters such as 5-HT and NA. It is these transmitters that transmit the signal from one neuron to the other. It is important to understand the mechanisms underlying the transmission and reuptake inhibition of neurotransmitters. Both neurotransmitters are synthesised in the neuron cell body. Once they are synthesised, they rapidly move along the axon to nerve terminals by fast axonal transport. Vesicles store the neurotransmitters away from the cytoplasm in order to protect them from intra-cellular degradative enzymes such as monoamine oxidases. Vesicles are able to actively take up the neurotransmitters by a pH gradient. The vesicle's transport mechanism is activated by the hydrolysis of adenosine triphosphate (ATP). This hydrolysis increases the amount of protons in the vesicle resulting in a more acidic environment than that of the cytoplasm. This generates a positive energy potential. The neurotransmitter is then translocated into the vesicle by carrier-mediated transport. Serotonin and NA are accumulated in the vesicles. When a signal is needed, the synaptic vesicles attach themselves to the presynaptic membrane, then break open and spill neurotransmitters into the synaptic cleft, a process known as exocytosis. Once the signal needs to be ended, the neurotransmitter is disposed of by the neuron in one of three ways namely diffusion, enzymatic degradation, and reuptake. Diffusion removes a fraction of all chemical messengers into the extracellular fluid. Enzymatic degradation has many pathways within the neuron and in non-neural tissues. The enzymes can help to control the concentration of neurotransmitters in the neuron or in degrading those that have escaped.

The reuptake of the neurotransmitter from the synaptic cleft is the most common mechanism for inactivation. There is a high affinity uptake mechanism for the released neurotransmitter at the nerve endings (Schwartz, 1985). Certain drugs, for example 5-HT agonists or NA agonists, are effective because they either promote the release or inhibit the reuptake of 5-HT or NA, respectively (Garattini, 1995). The application of such drugs to block the uptake or to promote the release of these neurotransmitters prolongs and enhances their actions.

2.2.3-Effect of Diet on Brain Serotonin and Noradrenaline

The explanation for macronutrient differences was stated to be a result of the ratio of tryptophan, an essential amino acid that is the dietary precursor to 5-HT, to other large neutral amino acids (LNAA) namely: valine, isoleucine, leucine, tyrosine, methionine, and phenylalanine. Tryptophan must compete with the other LNAAs for transport across the blood-brain barrier (BBB). The ratio of tryptophan to the other LNAAs is crucial in establishing the amount of tryptophan present in the brain that is converted to 5-HT (refer to appendix A2 for 5-HT synthesis diagram). A carbohydrate-rich meal stimulates insulin secretion, which causes an uptake of amino acids from the blood into muscle with the exception of tryptophan since it is bound to albumin, which inhibits its uptake into the muscle. When bound to albumin, tryptophan competes with free fatty acids at the binding site. The presence of insulin facilitates the binding of free fatty acids to albumin thus releasing the tryptophan (reviewed by Strüder & Weicker, 2001). When this occurs, there is an increase in tryptophan relative to the other LNAAs in the blood ready to be transported across the BBB into the brain. This results in an increase in brain tryptophan, which facilitates 5-HT synthesis. Also, the rate-limiting enzyme in the synthesis of 5-HT

is tryptophan hydroxylase, which is unsaturated at physiological concentrations of tryptophan. Hence, an increase in tryptophan will increase the synthesis of 5-HT (Ashcroft et al., 1965). Ashcroft et al. hypothesised that this increase in 5-HT synthesis would provide feedback about recent nutrient intake and thus cause a decrease in carbohydrate consumption. A protein-rich meal also causes an increase in insulin secretion but provides exogenous amino acids to the blood. This leads to an overall increase in all amino acids in the blood with tyrosine being the most abundant and hence, tyrosine will be favoured in the competition for transport at the BBB (Fernstrom &Wurtman, 1971). Tyrosine, a semi-essential amino acid, is the precursor to dopamine, hence an increase in brain tyrosine may increase the synthesis of dopamine and NA (Wurtman et al., 1981). In fact, the concentration of tyrosine in the brain has been found to rise as high as two- to threefold when dietary protein intake is between 0-10% of the diet in the rat model. This increases the rate of catecholamine synthesis, namely for dopamine and NA, in the hypothalamus, which has been identified as an area of the brain involved in appetite regulation (as reviewed by Fernstrom and Fernstrom, 2001).

2.2.4-Sibutramine, Food Intake and Macronutrient Selection

Researchers have focused on sibutramine's effect on overall food intake and how it relates to weight loss. Sibutramine's main effect is as an appetite suppressant, leading to a decrease in food intake and a feeling of satiety (Fantino et al., 1995; Stricker-Krongrad et al, 1995). Sibutramine has also been shown to increase energy expenditure by enhancing thermogenesis (Stock, 1997; Connoley et al., 1999). Jackson et al. (1997a) examined the effect of sibutramine on cumulative food intake of freely-feeding adult

male Sprague-Dawley rats (350-500 g) over an 8-h period following the onset of the dark phase. The animals were conditioned to a reverse phase light-dark cycle with light on at 17h00 until 09h00 and had free access to a standard powdered rat diet. A 72-h washout period was used in-between experiments. Sibutramine, at doses 3 and 10mg/kg, diluted in deionized water, fed orally to the rats, produced a significant, dose-dependent decrease in food intake, which became evident within the first 2-h post-administration and continued over the 8-h measuring period.

Jackson et al. (1997b) conducted a similar experiment examining the mechanisms underlying the hypophagic effects of sibutramine. Freely-feeding adult male Sprague-Dawley rats (350-500 g) were conditioned to the same reverse light-dark cycle as in Jackson et al. (1997a) with free access to a standard powdered rat diet. The rats were fed 10mg/kg of sibutramine orally, diluted in deionized water, and their food intake was measured at 2, 4 and 8-h post-administration. Once again, sibutramine induced hypophagia at all measurement intervals. At the time of administration, prazosin, an α_1-adrenoreceptor antagonist, was also administered and significantly inhibited the effects of sibutramine on food intake in a dose-dependent manner. When metoprolol, a β_1-adrenoceptor antagonist, was administered at the same time as sibutramine it only partially inhibited the decrease in food intake. An α_2-adrenoreceptor antagonist, RX821002 and a β_2-adrenoceptor antagonist, ICI 118,551, did not antagonise the hypophagic effect caused by sibutramine. The 5-HT antagonists metergoline (non-selective), ritanserin (5-$HT_{2A/2C}$-receptor) and the 5-$HT_{2B/2C}$ -receptor antagonist SB200646 only partially inhibited the decrease in food intake produced by sibutramine

and this effect was only evident 8-h post-administration. None of the antagonists used in this experiment had an effect on food when administered in the absence of sibutramine. These results indicate that β_1-adrenoceptors, 5-$HT_{2A/2C}$-receptors and particularly α_1-adrenoreceptors are involved in the inhibitory effects of sibutramine on food intake.

Day and Bailey (1998) examined the effect of sibutramine on hyperphagic, obese-diabetic, leptin-defective ob/ob mice (sex was not reported). The mice were maintained on a fixed 12:12-h light-dark cycle with lights on from 08h00-22h00 and supplied a standard pellet diet and tap water. Sibutramine, at a dose of 5mg/kg, was administered orally by gavage for six weeks and food intake was measured daily. Sibutramine reduced weight gain but did not significantly alter daily food intake over the six weeks period. Chronic administration of sibutramine also decreased non-esterified fatty acids concentrations, lowered hyperinsulinaemia and improved insulin resistance in the ob/ob mice. Also, to study the acute effect of sibutramine, mice aged 12 weeks (sex not reported) were administered either 5mg/kg of sibutramine or placebo. Food was withheld from 0-8-h and then the mice were given free access to the food from 8-24-h and total intake was noted. Overall food intake was significantly reduced with the sibutramine dose with no significant effects noted for plasma glucose, insulin and non-esterified fatty acids concentrations over the 24-h period measured immediately before and 2, 4, 8, and 24-h after drug administration.

Grignaschi et al. (1999) conducted a study examining the effect of sibutramine in various feeding paradigms in rats. The rats were either food-deprived, neuropeptide Y

(NPY) or muscimol-injected. These three paradigms caused food overconsumption. Male Sprague-Dawley rats were kept in a 12:12-h light-dark cycle with lights off at 18h00 and provided water and food pellets *ad libitum*. In the 24-h food-deprived rats, sibutramine, at doses 1, 1.7, 3, 5.6, and 10mg/kg, was given orally 60 minutes before the presentation of food. Receptor antagonists such as metergoline (non-selective) was administered 3-h before the presentation of food, while ritanserin (5-$HT_{2A/2C}$ receptors), SB206553 (5-$HT_{2B/2C}$ receptors), or GR127935 (5-$HT_{1B/1D}$ receptors) were given 90 minutes before food was offered. The food intake was measured 1-h after presentation. In the NPY-injected rats, sibutramine, at doses 3, 4.1, 5.5, 7.4, and 10mg/kg, was also administered orally, 60 minutes before food was presented and the antagonist used was GR127935. For the muscimol-injected rats, sibutramine, at doses 1, 3 or 10mg/kg, was administered orally, 60 minutes before food was presented and the muscimol was injected 20 minutes before food was offered. Once again, the food intake was measured 1-h after presentation. Sibutramine reduced feeding in a dose-dependent manner for both the food-deprived and the NPY-injected rats. The increase in food intake caused by muscimol was not altered by sibutramine. With a dose of 5.1mg/kg of sibutramine, only the antagonist SB206553 slightly, yet significantly, reduced the effect of hypophagia in the food-deprived rats. Also, at 6.0mg/kg of sibutramine administered to NPY-injected rats, GR127935 did not modify the reduction in food intake. Having stated these results, it appears that there is a partial involvement of 5-$HT_{2B/2C}$ receptors in the hypophagic effect caused by sibutramine.

Brown et al. (2001) examined the effects of sibutramine on feeding and body weight in a rat model of dietary obesity. Male Wistar rats were conditioned to a reversed phase lighting 12:12-h light-dark cycle with lights on at 03h00. The rats were divided into two groups. One group was fed a highly palatable diet (33% chow, 33% Nestlé milk, and 7% sucrose) resulting in dietary-induced obesity and the other group was placed on standard pelleted chow for 12 weeks. Rats were then given by gavage, either 3mg/kg of sibutramine or deionized water for a period of 21 days. Sibutramine significantly reduced total food intake and body weight in both dietary-obese and chow-fed rats. The effects were more pronounced in the dietary-obese rats. A decrease in food intake was evident at 2-h post-administration and a significant weight loss was observed after day 2 in the dietary-obese rats. Strack et al. (2002) conducted a similar experiment examining whether sibutramine has increased efficacy in rats with dietary-induced obesity. Male Sprague-Dawley rats, maintained on a 12:12-h light-dark cycle (time not specified), were fed a moderate high-fat diet (16.8% protein, 51.4% carbohydrate, and 31% fat; source of macronutrients was not reported) from 3 weeks of age until either 8 weeks or 16 weeks of age. The rats were given orally (method not reported) either the vehicle or sibutramine at dose 0.6 or 2mg/kg for a period of 14 days. Daily food intake was measured at 4-h intervals. In the younger group, with 15% body fat, food intake was not significantly decreased by sibutramine. However, in the older group with 25% body fat, sibutramine decreased food intake in a dose-dependent manner with cumulative food intake decreasing significantly over the 14-day period. Despite this differential effect, sibutramine produced relative reductions in fat mass and had no effect on lean mass in both groups.

Since sibutramine is a relatively new drug, most studies to date have focused on its effectiveness in promoting and sustaining weight-loss in both animal and human models (reviewed by Luque & Rey, 2002). Only two human trials have examined sibutramine's possible effects on food intake. An American study by Rolls et al. (1998) examined the effect of daily dosing of 10mg or 30mg of sibutramine, taken immediately before breakfast, for a 2-week period, on overall total daily caloric intake. The daily total intake was measured on days 7 and 14 of the dosing period in a laboratory, in non-dieting moderately obese premenopausal women, aged 26 to 45. On the laboratory days, breakfast, lunch, and dinner were individual, buffet-style, attendant-served, self-selection meals from a variety of meal-appropriate and familiar foods. It was noted that with 30mg of sibutramine there was a significant increase in the percentage of energy consumed from carbohydrate and a reciprocal decrease in energy from fat. A French study by Chapelot et al. (2000) examined the effect of 15mg of sibutramine, administered at 08h30, on macronutrient selection in young male subjects, aged 18-25 years with a body mass index within the range of 18-25 kg/m^2 who were non-smokers, non-alcohol drinkers, and non-dieters. Part of the inclusion criteria included that the subjects like at least 35 of the 37 food items available at the experimental meals and usually consume four structured meals per day (breakfast, lunch, afternoon snack, and dinner). Breakfast was considered as a fixed preload since peak plasma concentrations of active sibutramine is reached 3-h after a single dose of 15mg (Garratt et al., 1995, as cited in Chapelot et al., 2000). Each subject was allowed to compose their lunch, afternoon snack (the French "goûter"), and dinner meals from the available items. The study resulted in a specific

carbohydrate reduction at the dinner meal but the proportions of macronutrients in total daily energy intake were not changed.

The results from these two human studies contradict each other. The differences could be related to a number of factors. In the above studies, the subjects were offered whole foods (examples include blueberry muffin, potato chips, fruit yogurt, beef meat and potatoes, and spaghetti sauce) so macronutrient selection should be interpreted with caution. Although subjects had a choice of foods, each food containing more than one macronutrient, it was difficult to interpret an effect on specific macronutrient selection. Humans eat food for many reasons other than energy balance and any trends such as a reduction in carbohydrate intake can be the result of many confounding factors rather than due to the effect of sibutramine alone. The rat model allows for control of many environmental and emotional confounders that occur in humans. This allows for a physiological understanding of behaviour and the effect of a drug such as sibutramine.

Chapter 3

MANUSCRIPT

Effect of Sibutramine on Macronutrient Selection in Male and Female Rats

Effect of Sibutramine on Macronutrient Selection in Male and Female Rats

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ABSTRACT

LEBLANC, M. AND L. THIBAULT. Effect of sibutramine on macronutrient selection in male and female rats. PHYSIOL BEHAV 78(5) 000-000, 2003.- Sibutramine, a serotonin-noradrenaline reuptake inhibitor (SNRI), has been shown to be a safe and effective weight-loss drug. The purpose of the present study was to examine whether sibutramine has an effect on macronutrient selection in both female and male rats in addition to total food intake. Wistar rats of both sexes were divided into three groups and each group was offered a different set of three sensorily contrasting macronutrient-specific diets, each set including a carbohydrate-rich diet, a protein-rich diet and a fat-rich diet. Sibutramine (10mg/kg) was shown to consistently decrease carbohydrate and fat intake at all data points regardless of gender and diet. The effect of sibutramine on protein intake was diet and gender-specific. All doses of sibutramine (2.5, 5, and 10mg/kg) decreased total food intake regardless of gender and diet group beginning at 6-h post-administration. In conclusion, sibutramine affected macronutrient selection and emphasis on dietary recommendations, as well as appropriate dosage according to gender should be considered during therapy.

Keywords: Carbohydrate Fat Feeding Macronutrient selection Protein Sibutramine

Running Head: Sibutramine and Macronutrient Selection

INTRODUCTION

In the ongoing battle against obesity, scientists have been struggling to find a solution. Current nutritional guidelines recommend a low-calorie diet and exercise to promote a weight loss (Health Canada, 2000). However, after initial weight loss, motivation seems to lapse after the expected plateau in weight loss for the individual has been reached (Lean, 2001). This inevitable weight loss plateau has spurred scientists to search for novel drugs. Drugs such as fenfluramine and fluoxetine were suggested as possible solutions, however, their potentially serious adverse effects has caused them to be removed from the market (Cannistra et al, 1997; Connoly et al., 1997; Mark et al., 1997; Rothman & Bauman, 2002). Sibutramine hydrochloride monohydrate, initially developed as an anti-depressant, was found to cause weight loss in depressed, obese test subjects (Kelly et al., 1995). Since then, it has been shown that sibutramine is a safe and effective weight-loss drug that can be administered for up to 2 years without serious side effects (Bray et al., 1998; James et al., 2000). At present, sibutramine has been approved for use, as an appetite suppressant in the treatment of obesity, in nearly 40 countries since its official introduction in February 1998 by Knoll Pharmaceuticals, a subsidiary of Abbott Laboratories (Luque & Rey, 2002).

Since sibutramine is a relatively new drug, most studies to date have focused on its effectiveness in promoting and sustaining weight-loss in both the animal and human model (Jackson et al., 1997a, 1997b; Day & Bailey, 1998; Grignaschi, et al., 1999; Brown et al., 2001; Strack et al., 2002). Only two human trials have examined sibutramine's possible effects on macronutrient selection but their results are inconclusive (Rolls et al., 1998; Chapelot et al., 2000).

Thibault and Booth (1999) stated that if only a single set of sensorily discernible diets are used in a study, then the results do not provide evidence for nutrient selection. The effects of the drug may be a result of the actions on the sensory or motor systems in the rat rather than on its behaviour controlled by the macronutrients. In order to control for this effect the experimental design of the present study consisted of three diet sets that were similar in energy content but that differed considerably in sensory characteristics.

Sibutramine is a serotonin-noradrenaline reuptake inhibitor (SNRI). A reuptake inhibitor inhibits the neuronal uptake of neurotransmitters and prolongs the duration of responses to both exogenous and neuronal release, in this case, of serotonin (5-HT) and noradrenaline (NA). Sibutramine has also been shown to block the re-uptake of dopamine (DA) but at about a 3-fold lower potency when compared to 5-HT and NA (Luscombe et al., 1989). More recent studies suggest that sibutramine increases extracellular DA concentrations at similar levels to 5-HT in an animal model (Bacioglu & Wurtman, 2000; Rowley et al., 2000). Serotonin is recognised to have an influence on food intake and macronutrient selection (reviewed by Thibault & Booth, 1999; Mok et al., 2000). Noradrenaline has also been shown to have an effect on food intake that opposes the effects of 5-HT, but its effects on macronutrient selection are not clear (Tempel & Leibowitz, 1990; Currie, 1993; Thibault & Booth, 1999). Dopamine has been shown to influence food intake but, similar to NA, its effects on macronutrient selection are not clear (Yang et al., 1997; Thibault & Booth, 1999). Hence, it was expected that sibutramine, in addition to affecting total food intake, would have an effect on macronutrient selection. Also, in the rat model, there has been evidence to support a difference in male and female rat macronutrient selection (Nance et al, 1977; Bjorntorp

1989; Richard & Rivest, 1989; Leibowitz et al, 1991; Veyrat-Durebex et al., 1998). The purpose of the present study was therefore to examine whether sibutramine would have an effect on macronutrient selection in both female and male rats in addition to total food intake.

MATERIAL AND METHODS

Animals and Diets

Sixty-two adult male and female Wistar rats (Charles River's, St. Constant, Quebec), weighing between 200 and 225 grams were used. Rats were individually housed under controlled temperature and humidity, with a constant 12:12h dark-light schedule with lights on at 0700h. The experiment was conducted in the summer. The rats were randomly divided into three separate dietary groups, with 10-11 males and females in each group. Each group was offered a simultaneous choice of three diets: a carbohydrate-rich diet, a protein-rich diet and a fat-rich diet (Table 1). The groups differed in the type of carbohydrate, protein and fat offered. The first group was fed a carbohydrate-rich diet mainly made from dextrin and cornstarch, a protein-rich diet mostly composed of casein and a fat-rich diet made primarily from unsalted butter. The second group was fed a carbohydrate-rich diet with a cornstarch base, a protein-rich diet containing soy isolate, and a fat-rich diet made mainly from lard. The third group was fed a carbohydrate-rich diet with main ingredients being sucrose and cornstarch, a protein-rich diet with an egg protein base and a fat-rich diet consisting mainly of margarine. The energy density of the egg protein diet differed from the densities of the other protein diets since the egg solids have a higher caloric density at 5.8 kcal/g. Both casein and soy isolate are 4 kcal/g. The fat diets were also different in energy density since butter has 7.2 kcal/g, margarine has 7.3 kcal/g and lard has 9 kcal/g. The proportion of cellulose content was the same across both the protein and the carbohydrate diets whereas the fat diets had a greater proportion of cellulose in order to match the energy density of the carbohydrate- and protein-rich diets. All diets were offered in a granular

form except for the lard-rich fat diet, which was in a paste form. All diets, except lard, were prepared one day prior to use by mixing them with a fixed amount of water pre-determined by a pilot study and stirring in order to obtain a granular form. The diets were left to dry for 24 hours to allow the added water to evaporate. No water was added to the lard diet, as it was offered in paste form that was pre-prepared and kept at 4°C to maintain freshness. All fat diets were kept at 4°C until the day of consumption. The casein diet was kept at -18°C until made into a granular form and then stored at 4°C since casein is prone to mold at room temperature. The rats had *ad libitum* access to food and tap water. Each rat was given three spill-proof feeders, chosen at random, and these feeders were labelled so as to remain with the same rat for the duration of the experiment to prevent odour-based changes in food consumption. All rats were weighed each day at 1500h.

Experimental Design and Treatment

The rats were given ten days to adapt to the diets and the environment. For the first three days, the rats had access to the food *ad libitum* with new food being provided at 1500h each day. On the fourth day, the rats began adaptation to a 4-h food deprivation period from 1500h to 1900h in preparation for the pre-treatment food deprivation period. Food intakes were measured everyday at 0100h, 0700h, 1500h, and 2100h, using a Mettler PJ 3000 scale, in order to observe the food intake changes after treatment at 2-h, 6-h, 10-h and 20-h post-administration.

After the ten-day adaptation period, at the end of the 4-h food deprivation period, the rats received by gavage either saline or one of three different doses of sibutramine, dissolved in water, at the beginning of the dark phase. Once all rats had been gavaged,

the food was distributed accordingly. As determined from the literature and a pilot study, the doses of sibutramine tested were 2.5, 5, and 10mg/kg (Meridia®, Ross Laboratories Canada) with the dilution ratio being 0.5mg/ml, 1.25mg/ml and 2.5mg/ml of Meridia® respectively. The doses were administered in ascending order and a two-day washout period followed each gavage day to prevent any cumulative effect. On the first day of treatment, each rat, acting as their own control, was gavaged with 2ml of saline (0.85% NaCl) using Fisher BD 3ml SlipTip™ syringes and a Fisher 3-inch feeding needle. On the next day, each rat was gavaged with the first dose of sibutramine, (2.5mg/kg), dissolved in water and following the same procedure as the saline treatment. A two-day washout period followed before the next dose of sibutramine (5mg/kg) followed by the third dose (10mg/kg) and then 2 final washout days. Therefore, the doses of sibutramine were administered on days 2, 5 and 8 of the treatment period. The order of the rats was randomised for each treatment. The volume of sibutramine to be administered to each rat was determined by the weight of each rat measured at 1500h that same day.

Statistical Analyses

Changes in body weight were analysed with repeated measures ANOVA, with dietary group and gender as between subject factors and treatment (sibutramine doses: saline, 2.5, 5, and 10mg/kg) as within subject factor. Total daily and post-administration (2-h, 6-h, 12-h, and 20-h) intakes for each macronutrient were analysed separately using Repeated Measures ANOVA with dietary group and gender as between subject factors and treatment as the within subject factor. When the main effects were statistically significant, multiple comparisons were tested for significance using Tukey-Kramer's test.

Results are expressed as mean ± SEM with differences considered statistically significant at $p<0.05$. All statistical analyses were carried out using SAS 8.0.

RESULTS

BODY WEIGHT

Variations in daily body weight for male and female rats throughout the experiment period as well as variations in daily body weight within each diet group are shown in Figure 1. The main effects of diet and individual day were found to be significant ($F(2, 56) = 10.15$, $p=0.0002$ and $F(9, 504) = 103.54$, $p<0.0001$, respectively). There were also significant interactions between gender and day as well as diet and day ($F(9, 504) = 11.16$, $p<0.0001$ and $F(18, 504) = 5.37$, $p<0.0001$, respectively). The weight of the male rats, on any particular experiment day, was significantly different ($p<0.01$) from all previous days. The weight of the female rats, also on any particular experiment day, was significantly different ($p<0.05$) from the previous day with the exceptions between day 2 and 3, 8 and 9, and 9 and 10. The gender and day interaction also resulted in the male and female rats' weights being significantly different ($p<0.05$) only at experiment days 9 and 10. For the experiment period, the male rats gained an average of 4.94 ± 1.27 g per day and the female rats gained an average of 0.85 ± 1.26 g per day. Also, on the day following each treatment with sibutramine (day 3, 6, and 9), the male rats showed a significant weight loss ($p<0.01$). The female rats also consistently lost weight after each treatment day with sibutramine but the result was only significant on day 6 ($p<0.01$).

DIETARY INTAKE

Carbohydrate-Rich Diet Intake

Statistical significance of dose, diet, gender and their interactions on 2-h, 6-h, 12-h, and 20-h carbohydrate- (CHO) rich diet intake is shown in Table 2. The main effects of dose, diet, and gender were found to be statistically significant at all time intervals. All doses (2.5, 5, and 10mg/kg) significantly decreased intake of the CHO-rich diets at all time intervals (2-h, 6-h. 12-h, and 20-h) when compared to saline as shown in Figure 2. The effect of the highest dose (10mg/kg) was significantly different from the other lower doses (2.5 and 5 mg/kg) with the rats eating less at the highest dose. A significant interaction was only found at 2-h post-administration between dose and gender ($p=0.0142$) as shown in Figure 3. In fact, in male rats, the 10mg/kg dose of sibutramine significantly decreased ($p<0.0001$) CHO-rich diet intake 2-h post-administration when compared to saline and to the other two doses of 2.5 and 5 mg/kg. In female rats, all sibutramine doses significantly decreased (2.5mg/kg: $p<0.0001$; 5mg/kg: $p=0.0014$; 10mg/kg: $p<0.0001$) CHO-rich diet intake compared to saline. In addition, female rats ate significantly less CHO-rich diet than male rats at 2.5mg/kg ($p=0.0048$) and 5mg/kg ($p=0.0013$) doses. At the highest dose, 10mg/kg, both genders showed a similar reduction in CHO-rich diet intake.

Protein-Rich Diet Intake

Statistical significance of dose, diet, gender, and their interactions on 2-h, 6-h, 12-h, and 20-h protein- (PRO) rich diet intake is shown in Table 2. The main effects of dose, diet, and gender were found to be statistically significant at all time intervals. Significant interactions were found at all time intervals between dose and gender and

dose and diet. The effect of dose and diet as well as the effect of dose and gender on protein-rich (PRO-rich) diet intake at 2-h post-administration of either saline or sibutramine are presented in Figures 4. When examining the significant interaction between dose and diet at 2-h post-administration, the rats fed the casein-rich diet, when receiving 2.5 or 10mg/kg of sibutramine, decreased their protein intake compared to saline ($p=0.0063$ and $p<0.0001$, respectively). In rats fed the soy-isolate-rich diet, intake was decreased at all doses (2.5mg/kg: $p=0.0034$; 5 and 10mg/kg: $p<0.0001$) when compared to saline. No significant change in intake following sibutramine administration was observed in the rats fed the egg solids-rich diet. The results for the significant interaction between dose and gender at 2-h post-administration were that for male rats, all doses decreased PRO-rich diet intake compared to saline (2.5mg/kg: $p=0.002$; 5 and 10mg/kg: $p<0.0001$). In female rats, the 2.5 ($p=0.0413$) and 10 mg/kg ($p=0.0004$) doses decreased PRO-rich diet intake compared to saline. Furthermore, female rats ate less than male rats when given either saline ($p=0.0003$) or 2.5mg/kg ($p=0.0046$) of sibutramine.

At 6-h post-administration, the significant interaction between dose and diet resulted in that the rats fed the casein-rich diet had an intake that was decreased at all dose levels when compared to saline (2.5mg/kg: $p=0.0032$; 5mg/kg: $p=0.039$; 10mg/kg: $p<0.0001$). Rats fed the soy isolate-rich diet also had an intake that was decreased at all dose levels when compared to saline (2.5mg/kg: $p=0.0027$; 5 and 10mg/kg: $p<0.0001$). However, in rats fed the egg solids-rich diet, only the highest dose (10mg/kg) decreased the PRO-rich diet intake when compared to saline ($p=0.0006$). The interaction between gender and dose at 6-h, 12-h, and 20-h post-administration indicated that for the male

rats, the PRO-rich diet intake was decreased significantly by all doses and the statistical significance increased as the dose of sibutramine increased suggesting a true dose response relationship. For the female rats, the PRO-rich diet intake was significantly decreased at all doses when compared to saline.

Fat-Rich Diet Intake

The effect of dose on fat-rich diet intake at 2-h, 6-h, 12-h, and 20-h following the administration of saline or sibutramine is represented in Figure 5 and statistical significance of dose, diet, gender, and their interactions is shown in Table 3. The main effects of dose and diet were found to be significant at all time intervals while the gender effect was only statistically significant at 20-h post-administration. When examining the effect of dose at 2-h post-administration, only the 10mg/kg dose was found to significantly decrease fat-rich diet intake when compared to saline ($p=0.005$). At 6-h post-administration, the effect of dose resulted in that both the 2.5 ($p=0.0067$) and 10mg/kg ($p=0.0005$) doses decreased fat-rich diet intake significantly when compared to saline. The effect of dose at 12-h post-administration was that the highest dose of 10mg/kg significantly decreased the intake when compared to saline ($p=0.0047$). At 20-h post-administration, the effect of dose resulted in that only the highest dose of 10mg/kg ($p=0.0157$) significantly decreased intake when compared to saline.

Total Intake

Statistical significance of dose, diet, gender, and their interactions on 2-h, 6-h, 12-h, and 20-h post-administration is shown in Table 3. The main effects of dose, diet, and gender were found to be significant at all data points. The effect of dose at 2-h, 6-h, 12-h, and 20-h post-administration is illustrated in Figure 6 showing that all doses of

sibutramine significantly decreased total food intake when compared to saline at all time intervals. The effects of dose and diet and dose and gender on total intake at 2-h post-administration are represented in Figures 7. A significant interaction was found between dose and diet at 2-h post-administration ($p=0.035$). For the diet group 1 (dextrin-cornstarch, casein, butter diets), doses 2.5 ($p=0.0007$) and 10mg/kg ($p<0.0001$) significantly decreased total intake when compared to saline. When examining the effect of the diet group 2 (cornstarch, soy-isolate, and lard diets), total intake significantly decreased by all doses when compared to saline. Total intake for diet group 3 (sucrose/cornstarch, egg solids, and margarine diets) decreased significantly for dose 10mg/kg only compared to saline ($p<0.0001$). When comparing the sibutramine doses alone, the 10mg/kg dose caused a significantly lower total intake when compared to the 2.5 and 5 mg/kg doses for both diet group 1 ($p<0.0001$) and 2 (2.5mg/kg: $p<0.0001$; 5mg/kg: $p=0.0016$) and when compared to the 5mg/kg ($p=0.0416$) dose for diet group 3.

Table 1. Composition of macronutrient diets (dry weights, %, g/100g)

Ingredients	Protein			Carbohydrate			Fat		
	P1	P2	P3	C1	C2	C3	F1	F2	F3
Vitamin free casein[b]	88.6								
Soy isolate[b]		88.6							
Egg solids[a]			88.6						
Cornstarch[b]				41.4	88.6	41.4			
Dextrin[a]				47.2					
Sucrose[b]						47.2			
Unsalted Butter (Zel)							48.78		
Lard (Tenderflake)								48.78	
Margarine (Becel)									48.78
Cellulose powder[b]	5.0	5.0	5.0	5.0	5.0	5.0	44.82	44.82	44.82
AIN76 mineral mixture[b]	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
AIN76 vitamin mixture[b]	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Choline chloride[b]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Energy density (kcal/g diet)	3.6	3.6	5.2	3.5	3.6	3.6	3.6	4.5	3.6

[a] Bio-Serv (Frenchtown, NJ)
[b] ICN Biomedicals (Aurora, OH)

Table 2. Three-way ANOVA results for carbohydrate-rich diet and protein-rich diet intake at 2-h, 6-h, 12-h, and 20-h post-administration (p.a.) of sibutramine.

	Carbohydrate-Rich Diet Intake				Protein-Rich Diet Intake			
EFFECT	**2-h p.a.**	**6-h p.a.**	**12-h p.a.**	**20-h p.a.**	**2-h p.a.**	**6-h p.a.**	**12-h p.a.**	**20-h p.a.**
Dose	F (3, 168) = 1.35, p<0.0001	F (3, 168) = 19.00, p<0.0001	F (3, 168) = 19.04 p<0.0001	F (3, 168) = 20.00, p<0.0001	F (3, 168) = 31.31, p<0.0001	F (3, 168) = 51.09, p<0.0001	F (3, 168) = 68.93, p<0.0001	F (3, 168) = 62.65, p<0.0001
Diet	F (2, 56) = 3.48, p=0.0375	F (2, 56) = 7.54, p=0.0013	F (2, 56) = 7.37, p=0.0014	F (2, 56) = 8.33, p=0.0007	F (2, 56) = 7.26, p=0.0016	F (2, 56) = 8.78, p=0.0005	F (2, 56) = 15.46, p<0.0001	F (2, 56) = 15.08, p<0.0001
Gender	F (1, 56) = 15.75, p=0.0002	F (1, 56) = 26.91, p<0.0001	F (1, 56) = 22.80, p<0.0001	F (1, 56) = 17.94, p<0.0001	F (1, 56) = 15.62, p=0.0002	F (1, 56) = 34.30, p<0.0001	F (1, 56) = 49.10, p<0.0001	F (1, 56) = 41.33, p<0.0001
Dose * Diet	F (6, 168) = 1.35, p=0.2380	F (6, 168) = 0.73, p=0.6281	F (6, 168) = 0.94, p=0.4687	F (6, 168) = 1.33, p=0.2460	F (6, 168) = 4.03, p=0.0009	F (6, 168) = 2.96, p=0.0089	F (6, 168) = 2.88, p=0.0106	F (6, 168) = 3.08, p=0.0069
Dose * Gender	F (3, 168) = 3.63, p=0.0142	F (3, 168) = 1.44, p=0.2328	F (3, 168) = 1.06, p=0.3658	F (3, 168) = 2.21, p=0.0892	F (3, 168) = 3.73, p=0.0125	F (3, 168) = 6.59, p=0.0003	F (3, 168) = 6.87, p=0.0002	F (3, 168) = 6.24, p=0.0005
Diet * Gender	F (2, 56) = 1.06, p=0.3525	F (2, 56) = 1.22, p=0.3020	F (2, 56) = 0.72, p=0.4910	F (2, 56) = 0.58, p=0.5623	F (2, 56) = 2.86, p=0.0658	F (2, 56) = 2.69, p=0.0769	F (2, 56) = 1.85, p=0.1667	F (2, 56) = 1.62, p=0.2062
Dose * Diet * Gender	F (6, 168) = 1.60, p=0.1505	F (6, 168) = 0.90, p=0.4947	F (6, 168) = 0.57, p=0.7513	F (6, 168) = 0.93, p=0.4750	F (6, 168) = 0.69, p=0.6612	F (6, 168) = 0.85, p=0.5328	F (6, 168) = 0.81, p=0.5649	F (6, 168) = 0.66, p=0.6782

Table 3. Three-way ANOVA results for fat-rich diet and total food intake 2-h, 6-h, 12-h, and 20-h post-administration (p.a.) of sibutramine.

	Fat-Rich Diet Intake				Total Food Intake			
EFFECT	**2-h p.a.**	**6-h p.a.**	**12-h p.a.**	**20-h p.a.**	**2-h p.a.**	**6-h p.a.**	**12-h p.a.**	**20-h p.a.**
Dose	F (3, 168) = 4.59, p=0.0041	F (3, 168) = 6.78, p=0.0002	F (3, 168) = 4.76, p=0.0033	F (3, 168) = 3.64, p=0.0141	F (3, 168) = 56.31, p<0.0001	F (3, 168) = 46.76, p<0.0001	F (3, 168) = 54.64, p<0.0001	F (3, 168) = 48.01, p<0.0001
Diet	F (2, 56) = 9.72, p=0.0002	F (2, 56) = 6.62, p=0.0026	F (2, 56) = 4.18, p=0.0203	F (2, 56) = 4.89, p=0.0110	F (2, 56) = 8.02, p=0.0009	F (2, 56) = 14.97, p<0.0001	F (2, 56) = 22.86, p<0.0001	F (2, 56) = 24.43, p<0.0001
Gender	F (1, 56) = 0.84, p=0.3634	F (1, 56) = 3.12, p=0.0830	F (1, 56) = 3.16, p=0.0809	F (1, 56) = 4.90, p=0.0310	F (1, 56) = 21.98, p<0.0001	F (1, 56) = 45.15, p<0.0001	F (1, 56) = 54.00, p<0.0001	F (1, 56) = 40.43, p<0.0001
Dose * Diet	F (6, 168) = 1.64, p=0.1396	F (6, 168) = 0.35, p=0.9093	F (6, 168) = 1.57 p=0.1583	F (6, 168) = 1.76, p=0.1112	F (6, 168) = 2.33, p=0.0350	F (6, 168) = 1.18, p=0.3205	F (6, 168) = 0.92, p=0.4792	F (6, 168) = 0.99, p=0.4309
Dose * Gender	F (3, 168) = 2.54, p=0.0582	F (3, 168) = 2.56, p=0.0570	F (3, 168) = 0.41, p=0.7467	F (3, 168) = 0.76, p=0.5168	F (3, 168) = 3.46, p=0.0178	F (3, 168) = 2.70, p=0.0471	F (3, 168) = 2.56, p=0.0566	F (3, 168) = 3.91, p=0.0099
Diet * Gender	F (2, 56) = 1.41, p=0.2527	F (2, 56) = 1.53, p=0.2253	F (2, 56) = 3.56, p=0.0350	F (2, 56) = 2.87, p=0.0652	F (2, 56) = 1.55, p=0.2206	F (2, 56) = 1.02, p=0.3688	F (2, 56) = 1.42, p=0.2511	F (2, 56) = 0.82, p=0.4435
Dose * Diet * Gender	F (6, 168) = 0.46, p=0.8377	F (6, 168) = 0.20, p=0.9764	F (6, 168) = 0.40, p=0.8763	F (6, 168) = 0.56, p=0.7648	F (6, 168) = 0.80, p=0.5691	F (6, 168) = 0.72, p=0.6361	F (6, 168) = 0.53 p=0.7882	F (6, 168) = 0.32, p=0.9267

Figure 1. Mean (± SEM) daily body weight (g) for male and female rats during the experimental period.

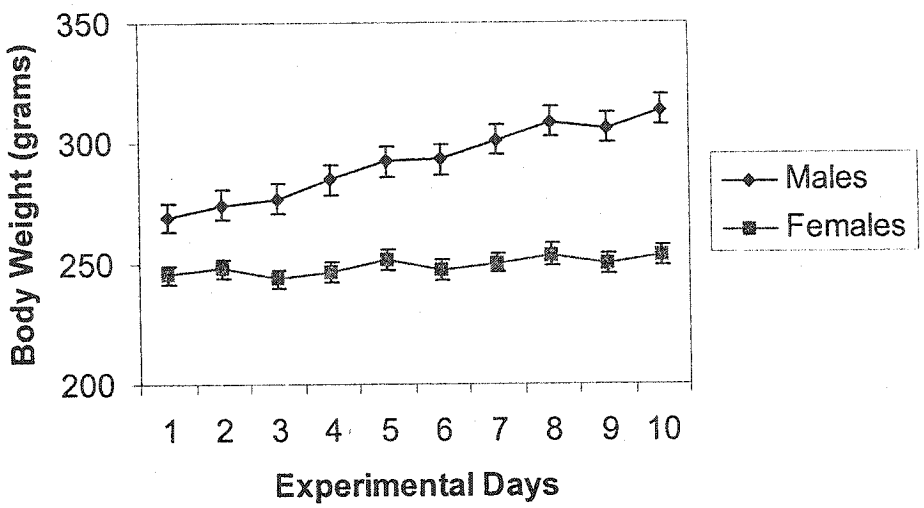
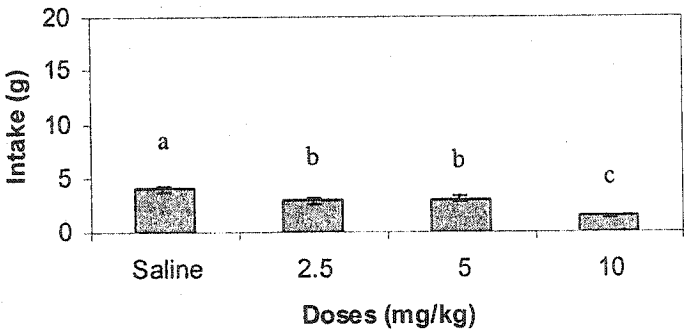


Figure 2. Mean (± SEM) carbohydrate-rich diet intake (g) at 2-h (A), 6-h (B), 12-h (C), and 20-h (D) following sibutramine administration. ‡

A



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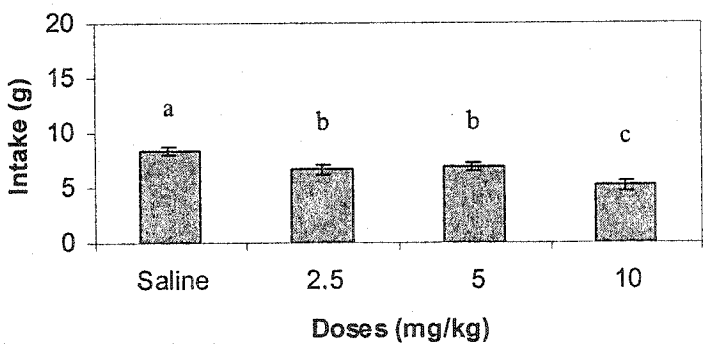

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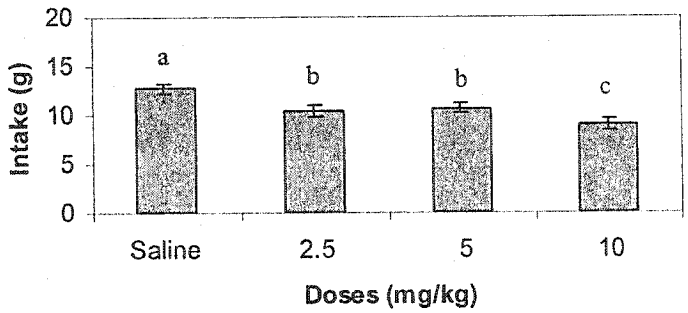

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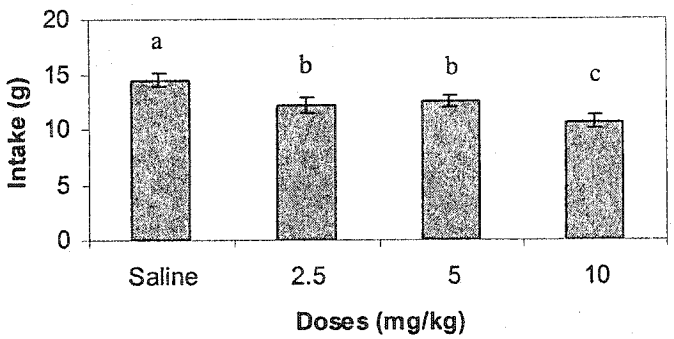
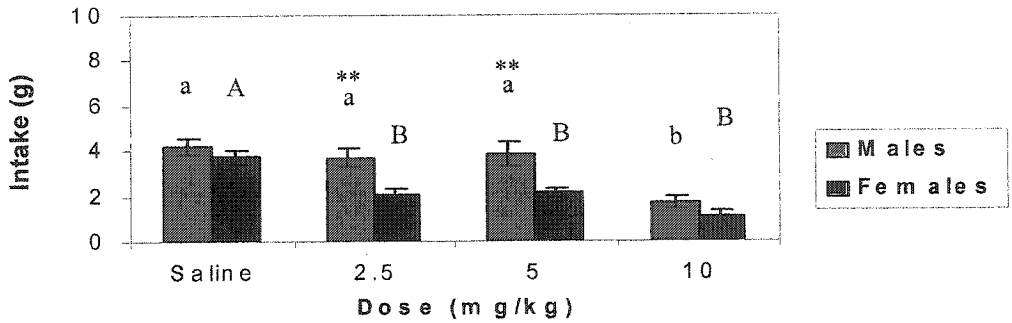


‡: Values with different letters are significantly different ($p<0.05$).

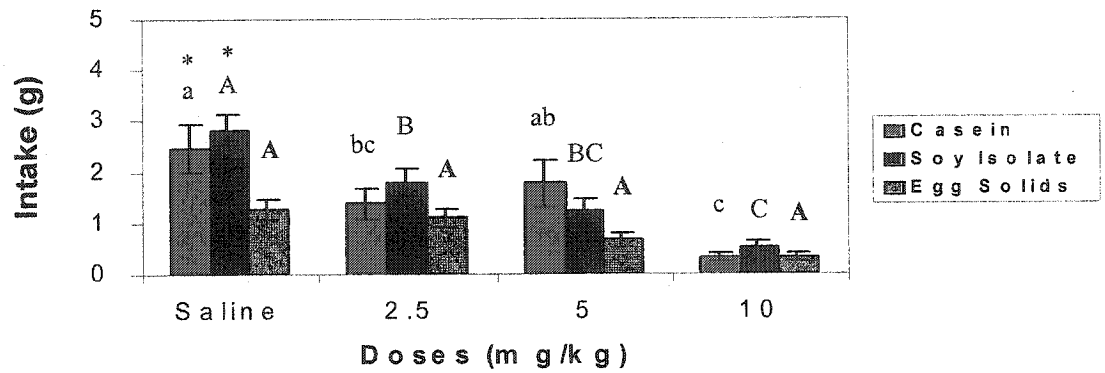
Figure 3. Mean (± SEM) carbohydrate-rich diet intake (g) for female and male rats in the 2-h following sibutramine administration. ‡



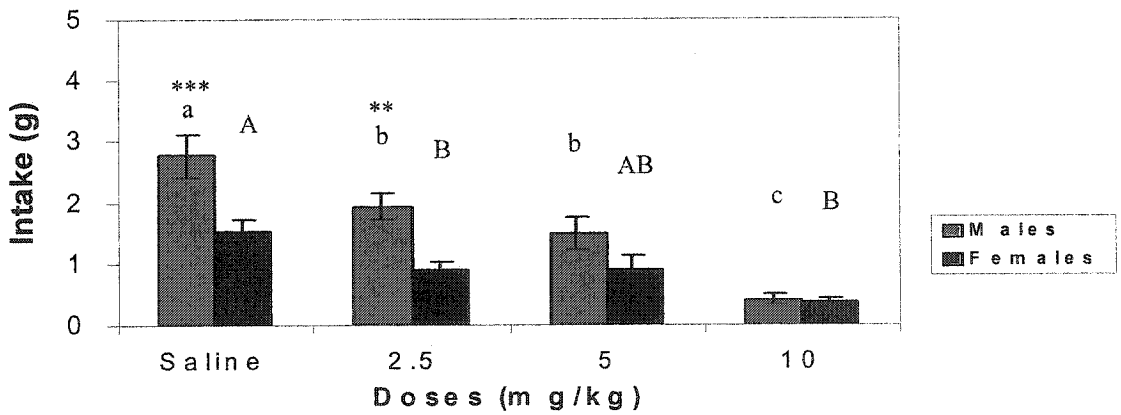
‡: Values with different letters are significantly different within gender ($p<0.01$).
**: Indicates a significant gender difference within a given dose ($p<0.01$).

Figure 4. Mean (± SEM) protein-rich diet intake (g) for each diet (casein, soy isolate, egg solids) (A) and for female and male rats (B) in the 2-h following sibutramine administration. ‡

A



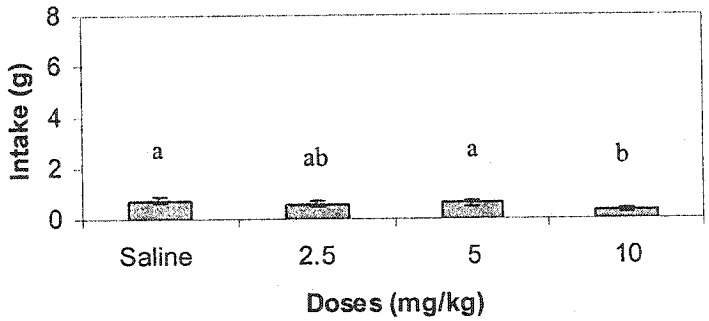
B



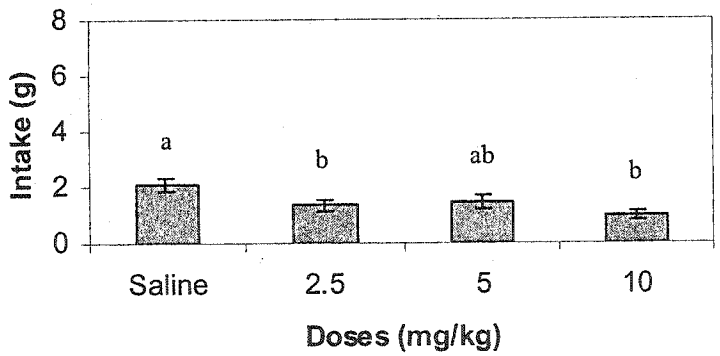
‡: Values with different letters are significantly different within diet groups or within gender ($p<0.05$).
*: Indicates a significant difference compared to egg solids-rich diet intake within dose ($p<0.05$).
**: Indicates a significant gender difference within a given dose ($p<0.01$).
***: Indicates a significant gender difference within a given dose ($p<0.001$).

Figure 5. Mean (± SEM) fat-rich diet intake (g) at 2-h (A), 6-h (B), 12-h (C), and 20-h (D) following sibutramine administration. ‡

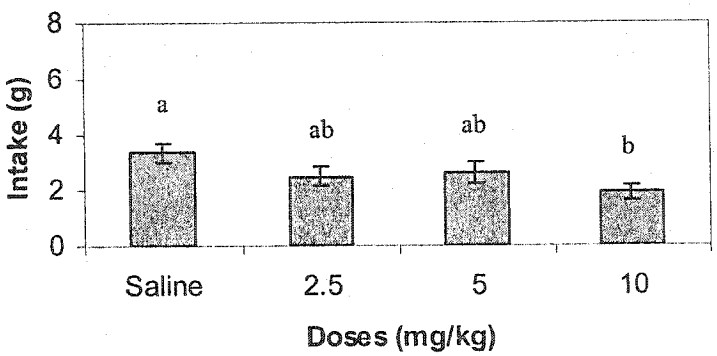
A



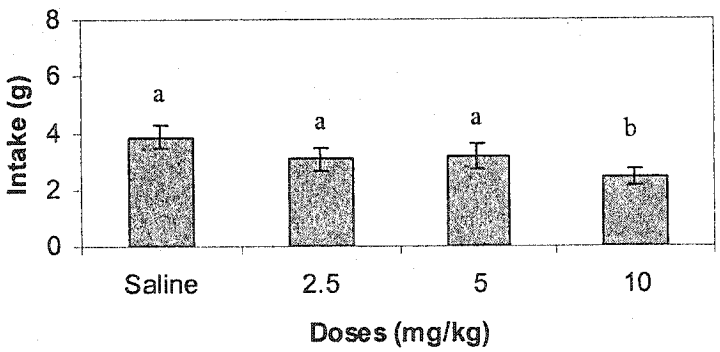
B



C



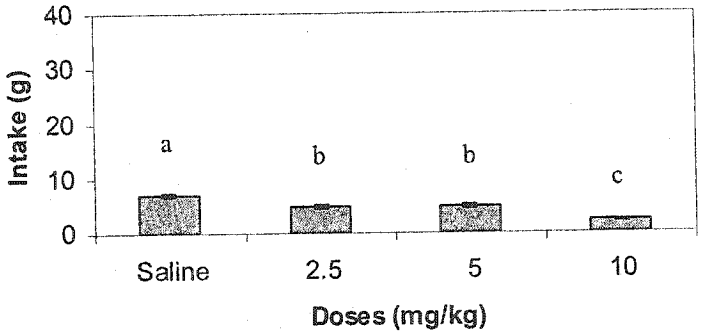
D



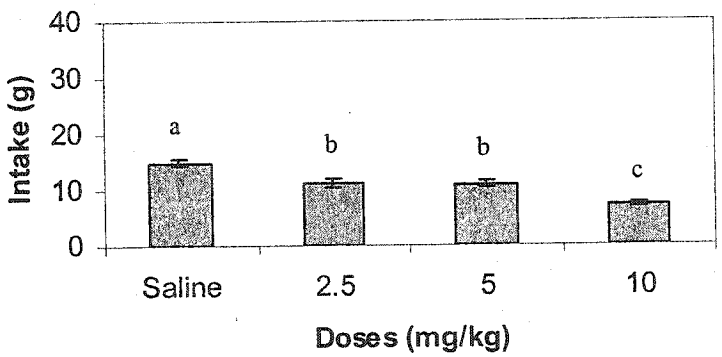
‡: Values with different letters are significantly different ($p<0.05$).

Figure 6. Mean (± SEM) total food intake (g) at 2-h (A), 6-h (B), 12-h (C), and 20-h (D) following sibutramine administration. ‡

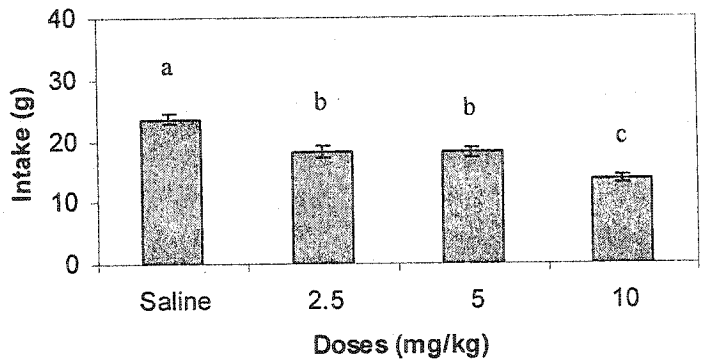
A



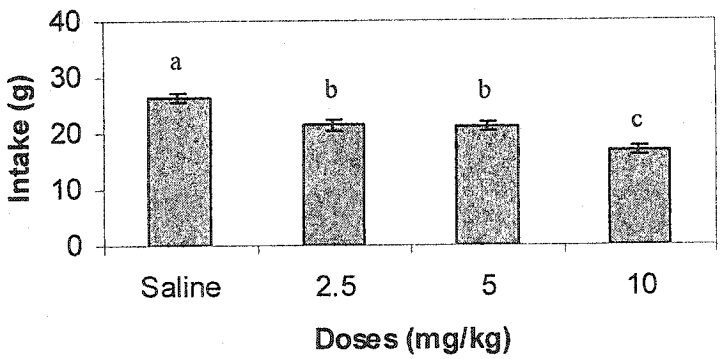
B



C



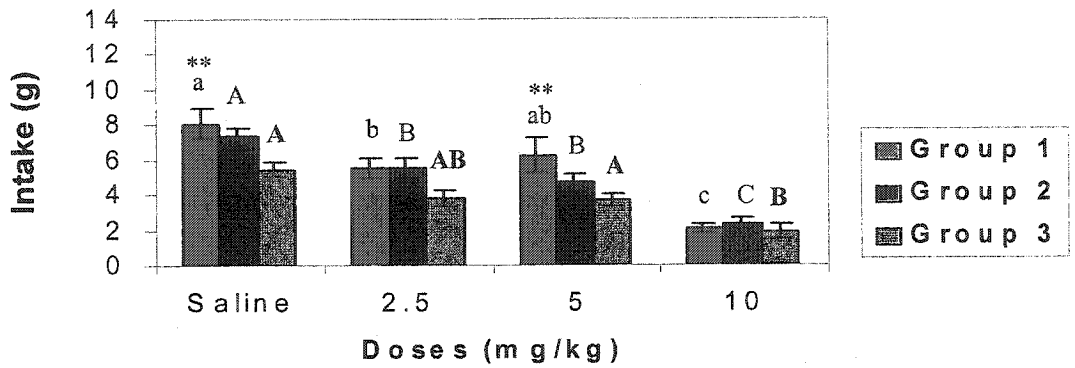
D



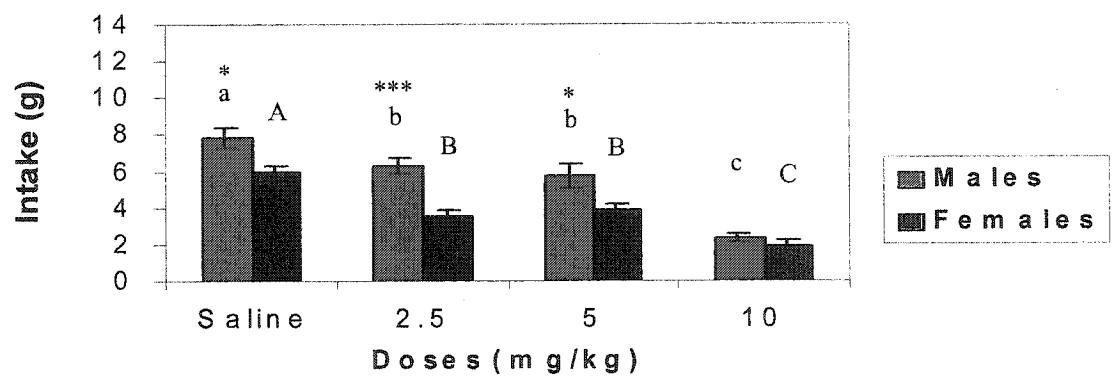
‡: Values with different letters are significantly different ($p<0.05$).

Figure 7. Mean (± SEM) total food intake (g) for groups 1, 2, and 3 (A) and for female and male rats (B) at 2-h following sibutramine administration. ‡†

A



B



‡: Values with different letters are significantly different within diet groups or within gender ($p<0.05$).
*: Indicates a significant gender difference within a given dose ($p<0.05$).
**: Indicates a significant difference compared to diet group 3 total intake within dose ($p<0.05$).
***: Indicates a significant gender difference within a given dose ($p<0.001$).
†: Group 1 (dextrin/cornstarch, casein, and butter); Group 2 (cornstarch, soy isolate, and lard); Group 3 (sucrose/cornstarch, egg solids, and margarine).

DISCUSSION

The administration of 2.5, 5, and 10mg/kg of sibutramine, to groups of adult male and female rats at the beginning of the dark phase, resulted in the highest dose (10mg/kg) consistently decreasing intake across carbohydrate-rich diets (dextrin/cornstarch, cornstarch, sucrose/cornstarch) in both male and female rats at all data points (2-h, 6-h, 12-h and 20-h post-administration). Since rats were offered sensorily contrasting sources of carbohydrate we can assume that this effect of sibutramine was nutritional, meaning specific to carbohydrate. Sibutramine was also shown to have a nutritional effect on fat by decreasing intake of fat-rich diets (butter, lard, and margarine) regardless of gender at the highest dose of 10mg/kg. The effect of sibutramine on the intake of protein-rich diets (casein, soy isolate, egg solids) was inconsistent across genders, doses, and the various diets, suggesting a sensory effect. Sibutramine consistently decreased total food intake beginning at 6-h post-administration with a robust effect seen at 10mg/kg.

Thibault and Booth (1999) stated that if only a single set of sensorily discernible diets are used in a study, then the results do not provide evidence for nutrient selection. The effects of the drug may be a result of the actions on the sensory or motor systems in the rat rather than on its behaviour controlled by the macronutrients. In other words, there is the possibility that food intake is influenced by the nutrient content of the diet, and not by the action of the drug on macronutrient selection. So far, sibutramine has only been tested with one set of diets, made up mainly of chow, in animal studies whereas human studies investigated food selection. In the present study, three sets of sensorily

contrasting macronutrient-specific diets were offered to three heterogeneous groups of rats in order to unconfound any sensory and nutritional effects.

Only two human trials have examined sibutramine's possible effects on food intake. An American study by Rolls et al. (1998) examined the effect of daily dosing of 10mg and 30mg of sibutramine, taken immediately before breakfast, for a 2-week period, on overall total daily caloric intake. The daily total intake was measured on days 7 and 14 of the dosing period in a laboratory, in non-dieting moderately obese premenopausal women, aged 26 to 45. On the laboratory days, breakfast, lunch, and dinner were individual, buffet-style, attendant-served, self-selection meals from a variety of meal-appropriate and familiar foods. It was noted that with 30mg of sibutramine there was a significant increase in the percentage of energy consumed from carbohydrate and a reciprocal decrease in energy from fat. A French study by Chapelot et al. (2000) examined the effect of 15mg of sibutramine, administered at 08h30, on macronutrient selection in young male subjects, aged 18-25 years with a body mass index within the range of 18-25 kg/m^2 who were non-smokers, non-alcohol drinkers, and non-dieters. Part of the inclusion criteria included that the subjects like at least 35 of the 37 food items available at the experimental meals and usually consumed four structured meals per day (breakfast, lunch, afternoon snack, and dinner). Breakfast was considered as a fixed preload since peak plasma concentrations of active sibutramine is reached 3-h after a single dose of 15mg (Garratt et al., 1995, as cited in Chapelot et al., 2000). Each subject was allowed to compose their lunch, afternoon snack (the French "gouter"), and dinner meals from the available items. The study resulted in a specific carbohydrate reduction at the dinner meal but the proportions of macronutrients in total daily energy intake were

not changed. In the present study sibutramine also reduced the intake of carbohydrate of lean rat subjects.

The results from these two human studies contradict each other. The differences could be related to a number of factors. In the above studies, the subjects were offered whole foods (examples include blueberry muffin, potato chips, fruit yogurt, beef meat and potatoes, and spaghetti sauce) so macronutrient selection should be interpreted with caution. Although subjects had a choice of foods, each food containing more than one macronutrient, it was difficult to interpret an effect on specific macronutrient selection. Humans eat food for many reasons other than energy balance and any trends such as a reduction in carbohydrate intake can be the result of many confounding factors rather than due to the effect of sibutramine alone. The rat model used in this study design allowed for control of many environmental and emotional confounders that occur in humans. The animal model, in this case, permitted for a physiological understanding of behaviour and the effect of sibutramine on macronutrient selection.

Sibutramine's effect on carbohydrate-rich diets intake with the 10mg/kg dose, at 2-h, 6-h, 12-h and 20-h post-administration, is consistent with the findings of studies on the effect of injecting 5-HT or its agonists, such as fenfluramine, fluoxetine, and quipazine, into the paraventricular nucleus (PVN) or peripherally, at the onset of the dark phase, on macronutrient selection from one set of carbohydrate, protein and fat-rich diets where the intake of carbohydrate was decreased (Wurtman &Wurtman, 1977; Shor-Posner et al., 1986; Kim & Wurtman, 1987; Leibowitz et al., 1989; Luo & Li, 1990; Weiss et al, 1990; 1991; Leibowitz et al., 1993). Mok et al. (2000) conducted a study with a similar design to our present study but using quipazine, a selective 5-HT_3 agonist.

Doses of 2.5, 5.0, and 7.5mg/kg of quipazine were injected intraperitoneally (IP) in adult male and female Wistar rats. Each group received a different set of three choices of sensorily contrasting sources of isocaloric macronutrient pure diets: Group 1 (92.24% casein, 92.9% cornstarch, and 48.78% safflower oil diets), Group 2 (92.9% egg protein, 72.26% cornstarch/20.64% sucrose, and 48.78% lard diets), Group 3 (92.24% casein hydrolysate, 91.9% maltose dextrin, and 48.78% butter diets). The result of that study was that the intake of cornstarch-containing diets was reduced in both male and female rats, suggesting a nutritional effect of quipazine.

One possible reason for sibutramine's effect on carbohydrate-rich diets being similar to the findings using 5-HT and its agonists lies in the fact that although sibutramine is a 5-HT-NA reuptake inhibitor it has been shown that medial hypothalamic 5-HT acts antagonistically with NA to suppress total food intake, in part by inhibiting the effects of NA (Currie & Coscina, 1994). Also, Luscombe et al. (1990) was able to show that sibutramine has a ranked order on inhibition such that inhibition of NA reuptake is greater than 5-HT reuptake and then inhibition of 5-HT reuptake is greater than that of DA. If the inhibition of NA reuptake is stronger than 5-HT, one possible result is that there is a much stronger relative antagonistic effect of 5-HT on the NA that is present in the hypothalamus. This would result in the possible effect of NA on macronutrient selection being overridden by the larger presence of 5-HT in the hypothalamus. Thus, in the present study, the suppressive effect of sibutramine on the intake of carbohydrate-rich diets is suggestive of a larger presence of 5-HT in the hypothalamus. However, measurements of 5-HT and NA levels would be needed to verify this assumption.

Rats consume most of their food during the active period of a diurnal cycle, and are able to regulate their intake in relation to their needs on a daily basis. Freely feeding adult male rats consume most of their intake at the beginning and the end of the dark period or active phase (Tempel et al., 1989). In terms of macronutrient selection, there is a circadian variation in which adult male rats seem to prefer a carbohydrate diet at the beginning of the dark phase with a shift towards protein and fat at the end of the dark phase (Tempel et al, 1989, Lax et al., 1998). Leibowitz et al. (1991) reported that adult female rats differed in their diurnal rhythm by consuming a larger portion of their carbohydrate diet and fat in the light phase when compared to male rats. The adult female rat thus consumed a significantly lower percentage of carbohydrate and fat in the dark period compared to the male rats. In our study, 10mg/kg of sibutramine consistently suppressed carbohydrate-rich diets intake across all diets (dextrin/cornstarch, cornstarch, and sucrose/cornstarch), data points, and genders at all time intervals (2-h, 6-h, 12-h, and 20-h). This means the effect does not seem to reflect rats' circadian macronutrient preferences. Although, this constitutes a nutritional effect of sibutramine on the intake of carbohydrate-rich diets, further investigation should include diets containing sugars other than cornstarch.

Sibutramine's suppressive effect on the intake of the fat-rich diets (butter-rich, lard-rich, margarine-rich) being consistent at the highest dose of 10mg/kg does not concur with previous research on 5-HT and macronutrient selection (reviewed by Thibault & Booth, 1999). So far, injection of 5-HT, along with its agonists fenfluramine, dexfenfluramine, d-fenfluramine and, fluoxetine, caused inconsistent effects on fat intake from either a choice between diets that were mixed such as high-carbohydrate, high-

protein, or high-fat diets or a choice between only three macronutrient-specific diets. In regards to the present study, we are able to conclude that unlike other serotonergic agents, a nutritional effect of the 5-HT-NA reuptake inhibitor, sibutramine suppresses fat-rich diets intake. Thibault and Booth (1999) have suggested that the pasty texture of fat-rich diets such as those containing lard when compared to the powdery texture of the protein- and carbohydrate-rich diets could necessitate more mastication. However, our design incorporated three sensorily different fat-rich diets (butter-rich, lard-rich, and margarine-rich) of various textures unconfounding sensory and nutritional effects and ruling out a possible motor effect of sibutramine.

When examining the results of sibutramine administration on the intake protein-rich diets, the inconsistent effect among the different diets could be because of a sensory effect of sibutramine on the rats' protein-rich diet intake rather than a nutritionally based effect. Our results concur with the absence of a consistent effect of 5-HT and its agonists on protein-rich diet intake (reviewed by Thibault & Booth, 1999).

Sibutramine's suppressive effect on total food intake was consistent for all dose levels, diets, and genders, beginning at 6-h post-administration. Although previous research on sibutramine also reported a suppressive effect on total food intake , it occurred as early as 2-h post-administration. For example, Jackson et al. (1997a) examined the effect of sibutramine on cumulative food intake of freely-feeding adult male Sprague-Dawley rats (350-500 g) over an 8-h period following the onset of the dark phase. The animals were conditioned to a reverse phase light-dark cycle with light on at 17h00 until 09h00 and had free access to a standard powdered rat diet. A 72-h washout period was used in-between experiments. Sibutramine, at doses 3 and 10mg/kg, fed

orally, diluted in deionized water, to the rats, produced a significant, dose-dependent decrease in food intake, which became evident within the first 2-h post-administration and continued over the 8-h measuring period. Jackson et al. (1997b) conducted a similar experiment examining the mechanisms underlying the hypophagic effects of sibutramine. Freely-feeding adult male Sprague-Dawley rats (350-500 g) were conditioned to the same reverse light-dark cycle as in the Jackson et al. study (1997a) with free access to a standard powdered rat diet. The rats were fed 10mg/kg of sibutramine orally, diluted in deionized water, and their food intake was measured at 2, 4 and 8-h post-administration. Once again, sibutramine induced hypophagia at all measurement intervals. Brown et al. (2001) examined the effects of sibutramine on feeding and body weight in a rat model of dietary obesity. Male Wistar rats were conditioned to a reversed phase lighting 12-h:12-h light-dark cycle with lights on at 03h00. The rats were divided into two groups: one group was fed a highly palatable diet (33% chow, 33% Nestlé milk, and 7% sucrose) resulting in dietary-induced obesity and the other group was placed on standard pelleted chow for 12 weeks. Rats were then given by gavage, either 3mg/kg of sibutramine or deionized water for a period of 21 days. Sibutramine significantly reduced total food intake and body weight in both dietary-obese and chow-fed rats. A decrease in food intake was evident at 2-h post-administration and a significant weight loss was observed after day 2 in the dietary-obese rats. In the present study, sibutramine consistently suppressed total food intake at 6-h post-administration. This may be due to the fact that rats were administered Meridia®, instead of pure sibutramine, which contains sibutramine along with other inactive ingredients such as microcrystalline cellulose which could have

delayed the absorption of the drug and thus increased the length of the T_{max} (T_{max} for sibutramine is 1.2-h following oral administration).

It has been shown that when NA acts upon the α_1-adrenoreceptors there is a decrease in total food intake (Leibowitz, 1988; Wellman & Davies, 1991). It has been suggested that the effects of decreasing total food intake by sibutramine could be linked to the β_1-adrenoceptors, 5-$HT_{2A/2C}$ -receptors and particularly α_1-adrenoreceptors (Jackson et al, 1997b). However, the effects of sibutramine have been linked to other receptors as well (Halford et al., 1995; Tecott et al., 1996; Bray, 2000).

In the present study, male and female rats' food intake differed according to sibutramine doses. For example, at 2-h post-administration, the lower doses (2.5 and 5mg/kg) had a stronger effect on the female rats' carbohydrate-rich diet intake than in male rats' intake indicating that female rats could be more sensitive to lower doses of sibutramine shortly after administration.

The rats in this study were subjected to a 4-h food deprivation period before sibutramine administration. Lax et al. (1998) indicated that rats expressed a preference for fat following a 3-h food deprivation period. Rats have also been observed to prefer both carbohydrate and fat after a 2-h food deprivation period (Tempel et al., 1989). Therefore, although the suppressive effect of sibutramine on carbohydrate and fat in 4-h food deprived rats seems to be robust, it should be further tested in non-deprived rats.

All three of the acute doses of sibutramine resulted in significant weight loss in the male rats the day following the gavage day but the weight loss was not always significant for the female rats. Female rats have been shown to have a better control over

energy intake and body weight, which could have accounted for the non-significant results (Nance et al, 1977; Bjorntorp 1989; Richard & Rivest, 1989).

Sibutramine, in the present study, was shown to have a robust nutritional effect on carbohydrate and fat by decreasing intake across various sources of carbohydrates and fats regardless of gender. The effect of sibutramine on protein intake was shown to be sensory as demonstrated by an inconsistent decrease in intake of the protein-rich diets according to the various diets. However, sibutramine consistently decreased total food intake. Future studies should be designed to examine the exact mechanisms behind the effect of sibutramine on macronutrient selection and how this relates to the reuptake inhibition of each neurotransmitter (5-HT, NA, and DA) and their respective receptors. In addition, feeding microstructure such as eating latency, meal size, and inter-meal intervals should be assessed. Also, the possible gender-specific sensitivity to different doses of sibutramine should be addressed. With sibutramine being used as part of a weight loss program in humans, it would be useful to monitor actual carbohydrate, fat, and protein intakes in order to maintain adequate requirements of all macronutrients. This study's experimental design could also be used as the model for future research on sibutramine's effect on macronutrient selection since we were successful in unconfounding sibutramine's sensory and nutritional effects.

Chapter 4

GENERAL CONCLUSION

Sibutramine, administered orally by gavage, at increasing doses of 2.5, 5, and 10mg/kg, to three groups of male and female rats offered three sets of sensorily different macronutrient-specific diets at the beginning of the dark phase resulted in the highest dose (10mg/kg) having a nutritional effect on carbohydrate by decreasing intake across carbohydrate-rich diets (dextrin/cornstarch, cornstarch, sucrose/cornstarch) regardless of gender and at all data points (2-h, 6-h, 12-h, and 20-h). Sibutramine was also shown to have a nutritional effect on fat by decreasing intake of fat-rich diets (butter, lard, and margarine) regardless of diet and gender at the highest dose of 10mg/kg at all data points. The effect of sibutramine on protein intake was shown to be sensory as demonstrated by an inconsistent decrease in intake of the protein-rich diets (casein, soy isolate, and egg solids) according to gender, dose, and the various diets. Consequently, sibutramine consistently decreased total food intake with a robust effect seen at 10 mg/kg.

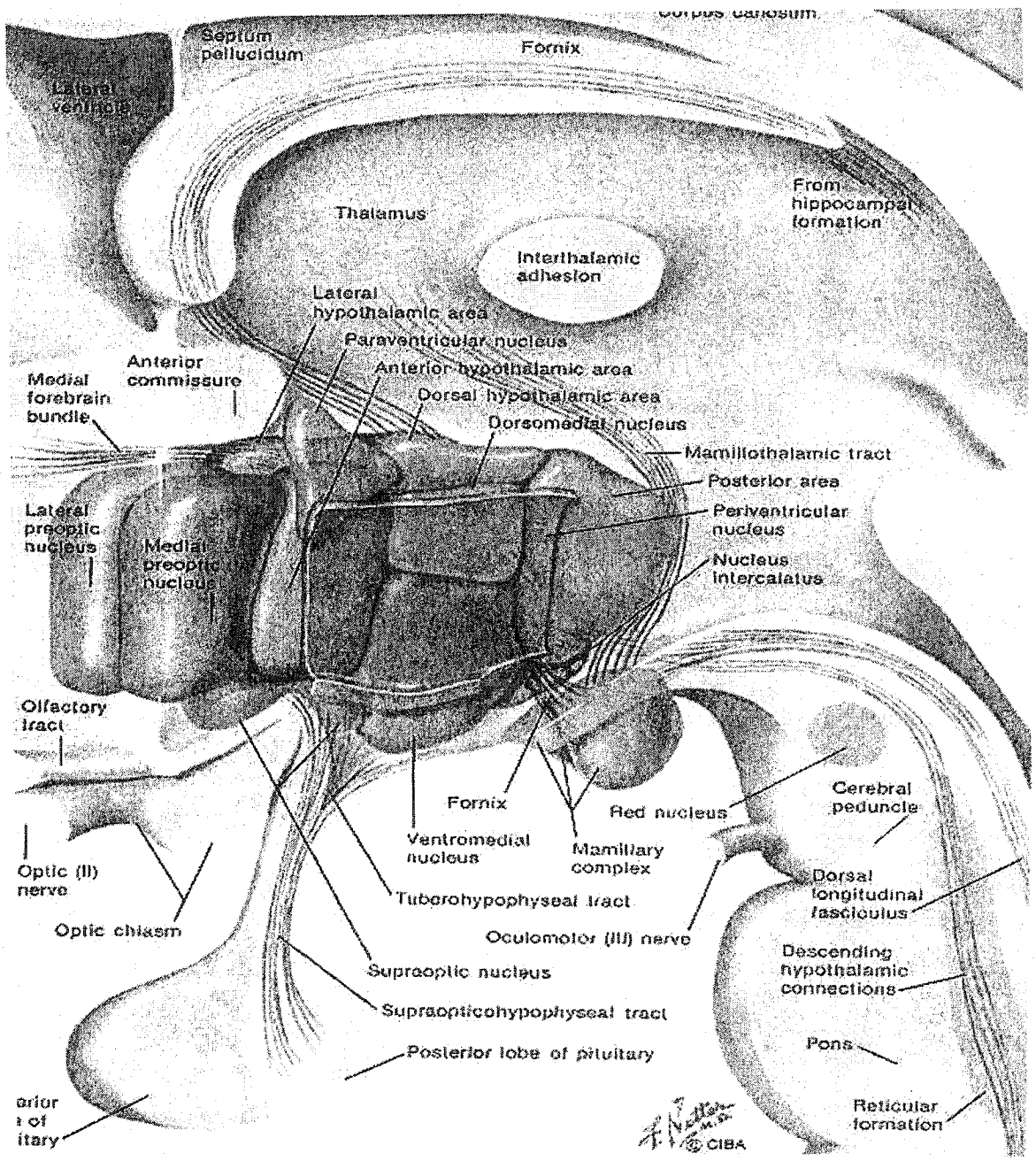
The results obtained in the present study of sibutramine having a suppressive carbohydrate effect concur with one human study by Chapelot et al., (2000) and with findings of studies using 5-HT or its agonists where the intake of carbohydrate was decreased (reviewed by Thibault & Booth, 1999). We suggested that this similarity in effect between sibutramine and 5-HT and its agonists was due to the fact that medial hypothalamic 5-HT was found to act antagonistically with NA to suppress total food intake. Also, we speculated that the suppressive effect of sibutramine on carbohydrate intake was due to a larger presence of 5-HT in the hypothalamus. This was explained by the fact that sibutramine has a ranked order on inhibition such that inhibition of NA

reuptake is greater than 5-HT reuptake which in turn is greater than the reuptake of DA. However, measurements of 5-HT and NA levels would be needed to verify this assumption. Although this intake suppression constitutes a nutritional effect of sibutramine using various sources of carbohydrate diets, further investigation should include research on sibutramine's effects on diets containing sugars other than cornstarch.

Sibutramine suppressed fat intake of varied fat sources, which also constitutes a nutritional effect. Such an effect does not concur with previous research. Our result of sibutramine having a suppressive effect on total food intake consistently for all dose levels, diets, and genders, began at 6-h post-administration. This concurs with previous research in which a consistent suppressive effect of sibutramine on total food intake was found. However, earlier work reported a decrease in total food intake as early as 2-h post-administration. We discussed the possibility that our result could be due to the fact that rats were administered Meridia®, instead of pure sibutramine, which could have delayed the absorption of the drug.

With sibutramine being used as part of a weight loss program in humans, it would be useful to monitor actual carbohydrate, fat, and protein intakes in order to maintain adequate requirements of all macronutrients. Also, this study's experimental design could be used as a model for future research on sibutramine's effect on macronutrient selection since we were successful in unconfounding sibutramine's sensory and nutritional effects.

Picture of the hypothalamus and its components, including the PVN, in humans



Source: www.psycheducation.org/emotion/ hypothalamus.htm

Serotonin Synthesis

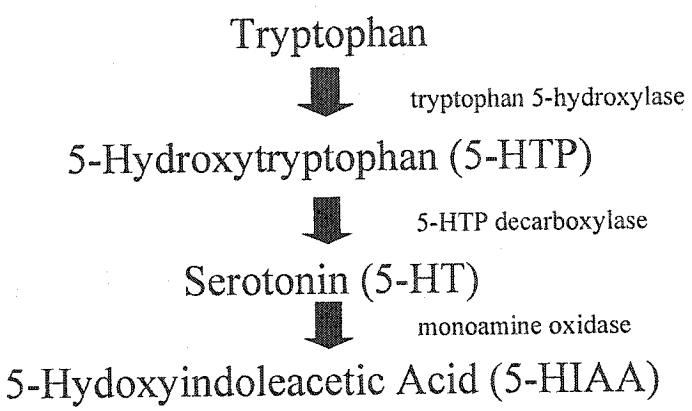


Table 1. Three-way ANOVA results for body weight.

EFFECT	*F value (df), P value*
Day	F (9, 504) = 103.54, p<0.0001
Diet	F (2, 56) = 10.15, p =0.0002
Gender	F (1, 56) = 1.05, p =0.3093
Day * Diet	F (18, 504) = 5.37, p<0.0001
Day * Gender	F (9, 504) = 11.16, p<0.0001
Diet * Gender	F (2, 56) = 1.42, p =0.2512
Day * Diet * Gender	F (18, 504) = 1.10, p =0.3490

Figure 1. Mean (± SEM) daily body weight (g) for each diet group during the experimental period.

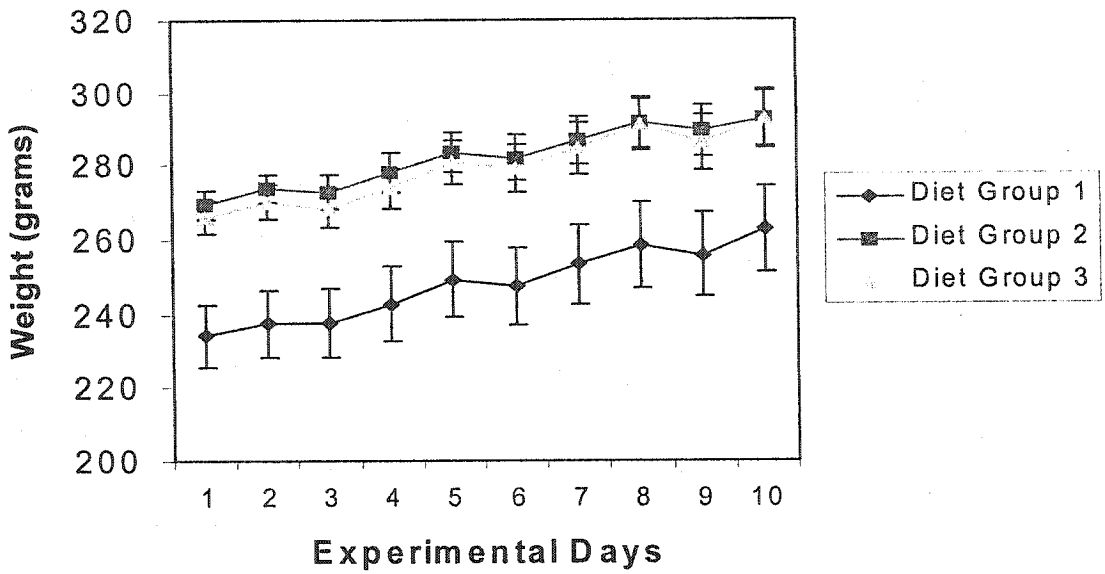
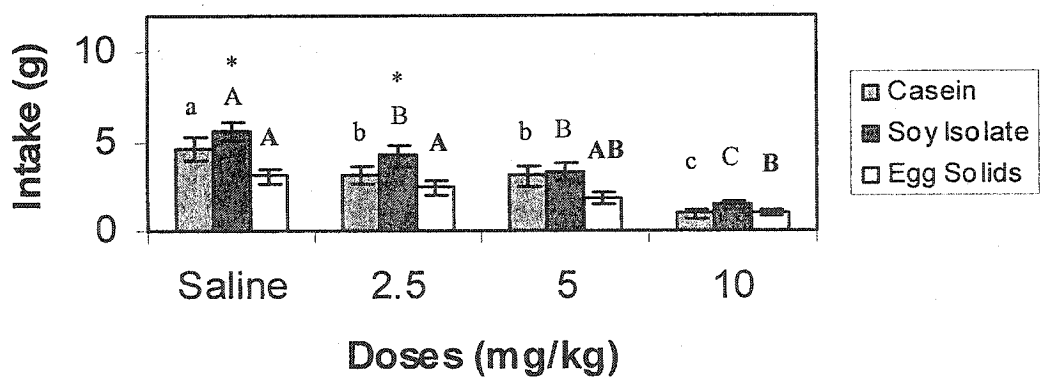
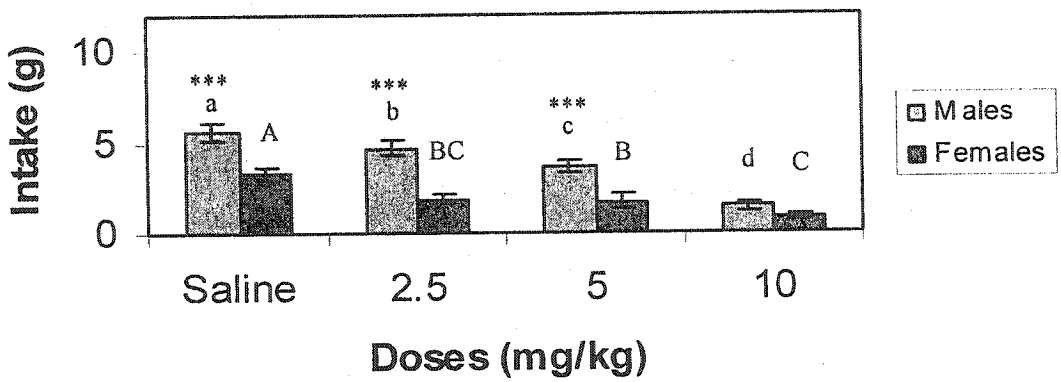


Figure 2. Mean (± SEM) protein-rich diet intake (g) for each diet (casein, soy isolate, egg solids) in the 6-h following sibutramine administration.‡



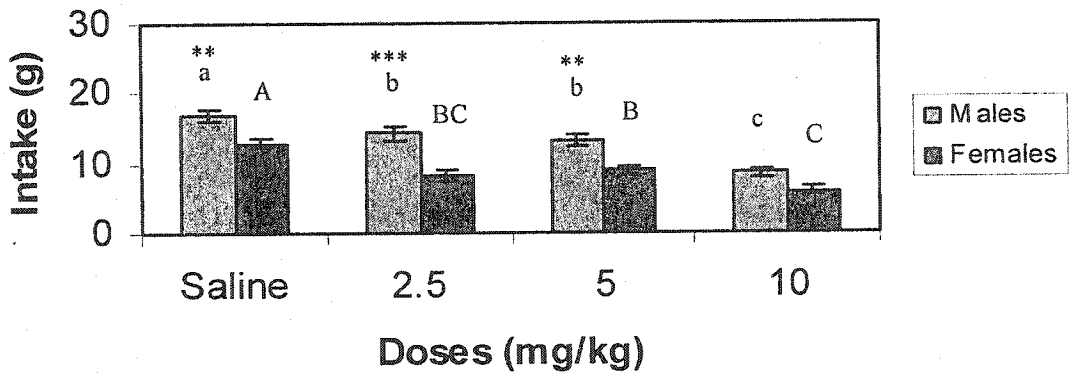
‡: Values with different letters are significantly different within each protein-rich diet ($p<0.05$).
*: Indicates a significant difference compared to Egg Solids-rich diet intake within dose ($p<0.05$).

Figure 3. Mean (± SEM) protein-rich diet intake (g) for female rats and male rats in the 6-h following sibutramine administration.‡



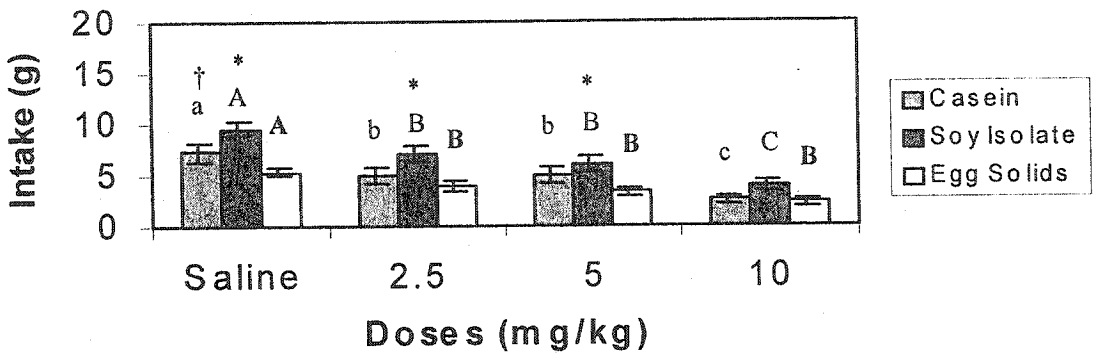
‡: Values with different letters are significantly different within gender ($p<0.05$).
***: Indicates a significant gender difference within a given dose ($p<0.001$).

Figure 4. Mean (± SEM) total food intake for female rats and male rats in the 6-h following sibutramine administration.‡



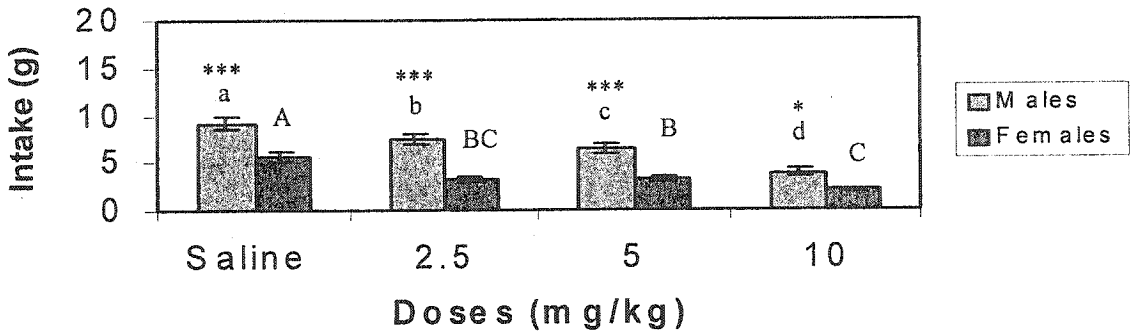
‡: Values with different letters are significantly different within gender ($p<0.05$).
**: Indicates a significant gender difference within a given dose ($p<0.01$).
***: Indicates a significant gender difference within a given dose ($p<0.001$).

Figure 5. Mean (± SEM) protein-rich diet intake (g) for each diet (casein, soy isolate, egg solids) in the 12-h following sibutramine administration.‡



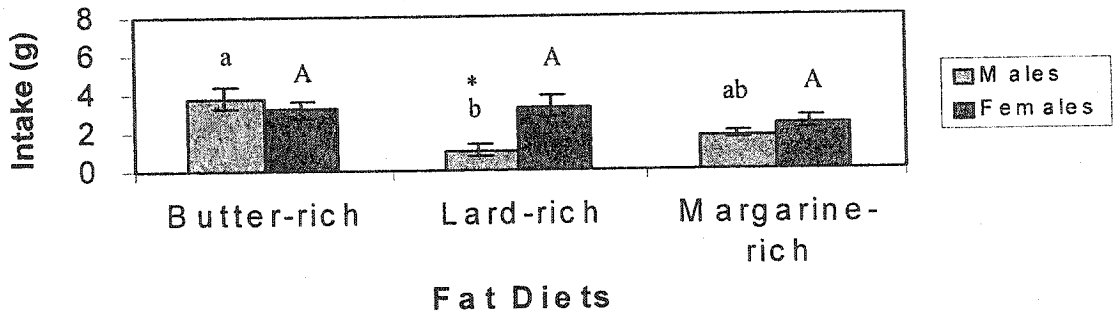
‡: Values with different letters are significantly different within each protein-rich diet ($p<0.05$).
†: Indicates a significant difference compared to Soy Isolate-rich diet intake within dose ($p<0.05$).
*: Indicates a significant difference compared to Egg Solids-rich diet intake within dose ($p<0.05$).

Figure 6. Mean (± SEM) protein-rich diet intake (g) for female rats and male rats in the 12-h following sibutramine administration.‡



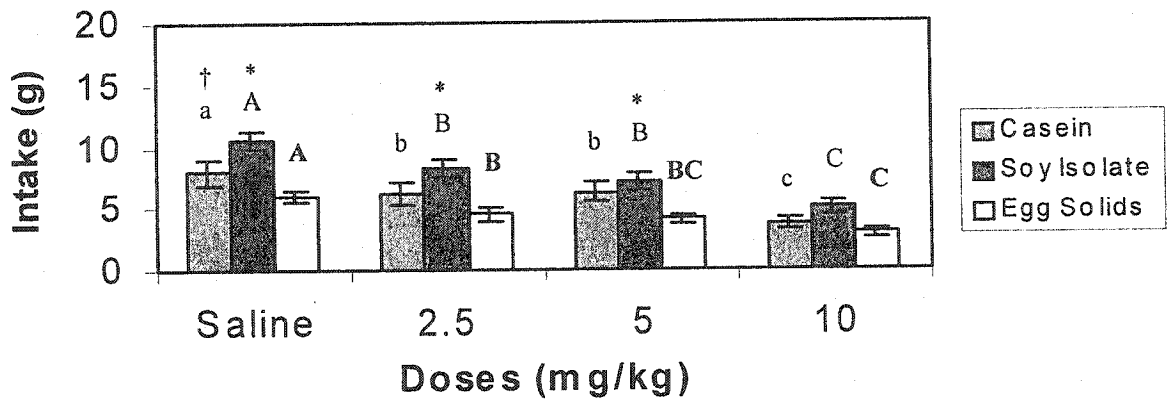
‡: Values with different letters are significantly different within gender ($p<0.05$).
*: Indicates a significant gender difference within a given dose ($p<0.05$).
***: Indicates a significant gender difference within a given dose ($p<0.001$).

Figure 7. Mean (± SEM) fat-rich diet intake (g) for each diet (butter, lard, and margarine) for female rats and male rats in the 12-h following sibutramine administration.‡



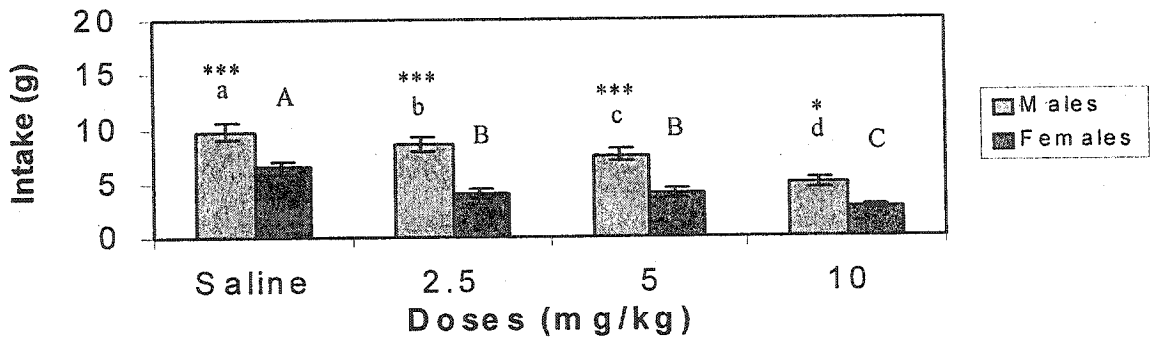
‡: Values with different letters are significantly different within gender ($p<0.05$).
*: Indicates a significant gender difference within a given diet ($p<0.05$).

Figure 8. Mean (± SEM) protein-rich diet intake (g) for each diet (casein, soy isolate, egg solids) in the 20-h following sibutramine administration.‡



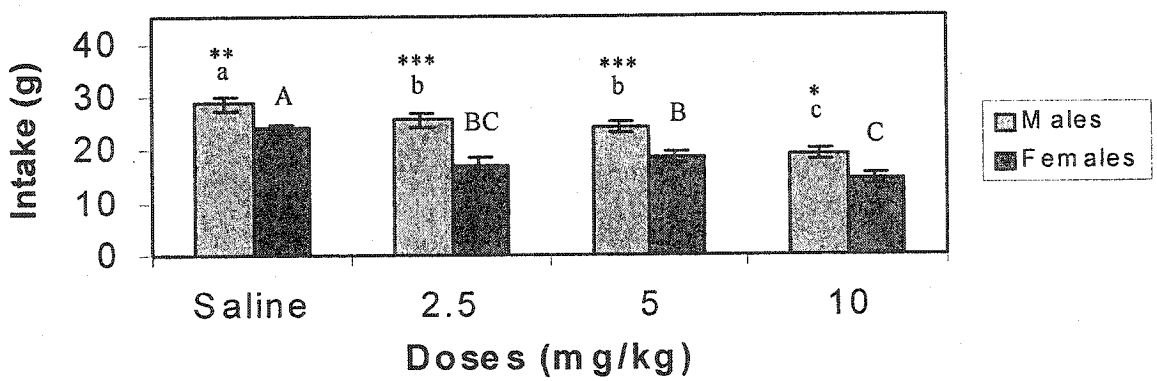
‡: Values with different letters are significantly different within each protein-rich diet ($p<0.05$).
†: Indicates a significant difference compared to Soy Isolate-rich diet intake within dose ($p<0.05$).
*: Indicates a significant difference compared to Egg Solids-rich diet intake within dose ($p<0.05$).

Figure 9. Mean (± SEM) protein-rich diet intake (g) for female rats and male rats in the 20-h following sibutramine administration.‡



‡: Values with different letters are significantly different within gender ($p<0.05$).
*: Indicates a significant gender difference within a given dose ($p<0.05$).
***: Indicates a significant gender difference within a given dose ($p<0.001$).

Figure 10. Mean (± SEM) total food intake (g) for female rats and male rats in the 20-h following sibutramine administration.‡



‡: Values with different letters are significantly different within gender ($p<0.05$).
*: Indicates a significant gender difference within a given dose ($p<0.05$).
**: Indicates a significance gender difference within a given dose ($p<0.01$).
***: Indicates a significant gender difference within a given dose ($p<0.001$).

B Lussier

A9

McGill University
Animal Use Protocol – Research

Guidelines for completing the form are available at www.mcgill.ca/fgsr/rgo/animal/

Protocol #: 4385
Investigator #: 537
Approval End Date: April 30, 2003
Facility Committee: AGR

__ Pilot __ New Application X Renewal of Protocol # 4385

Title (must match the title of the funding source application): Bases neurales du comportement ingestif et attributs sensoriels des nutriments

1. Investigator Data:

Principal Investigator: Louise Thibault, Ph.D. **Office #:**

Department: School of Dietetics and Human Nutrition **Fax#:**

Address: 21,111 Lakeshore Road, Ste-Anne-de-Bellevue **Email:**
Macdonald Campus, McGill University

2. Emergency Contacts: Two people must be designated to handle emergencies.

Name: Louise Thibault **Work #:** **Emergency #:**

Name: Emma Yiin **Work #:** **Emergency #:**

3. Funding Source:

External X	**Internal** __
Source (s): NSERC	**Source (s):**
Peer Reviewed: X YES __NO**	**Peer Reviewed:** __ YES __ NO**
Status : X Awarded __ Pending	**Status:** __ Awarded __ Pending
Funding period: April 2001-April 2006	**Funding period:**

** All projects that have not been peer reviewed for scientific merit by the funding source require 2 Peer Review Forms to be completed. e.g. Projects funded from industrial sources. Peer Review Forms are available at www.mcgill.ca/fgsr/rgo/animal/

Proposed Start Date of Animal Use (d/m/y): 1 April 2001 or ongoing __

Expected Date of Completion of Animal Use (d/m/y): 30 March 2006 or ongoing __

Investigator's Statement: The information in this application is exact and complete. I assure that all care and use of animals in this proposal will be in accordance with the guidelines and policies of the Canadian Council on Animal Care and those of McGill University. I shall request the Animal Care Committee's approval prior to any deviations from this protocol as approved. I understand that this approval is valid for one year and must be approved on an annual basis.

Principal Investigator: **Date:** 17 May 2002

Approval Signatures:

Chair, Facility Animal Care Committee:		**Date:** May 29, 2002
University Veterinarian:		**Date:** May 31, 2002
Chair, Ethics Subcommittee(as per UACC policy):		**Date:**
Approved Period for Animal Use	Beginning: MAY 1, 2002	Ending: April 30, 2003

This protocol has been approved with the modifications noted in Section 13.

JUN 06 2002

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